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TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1984

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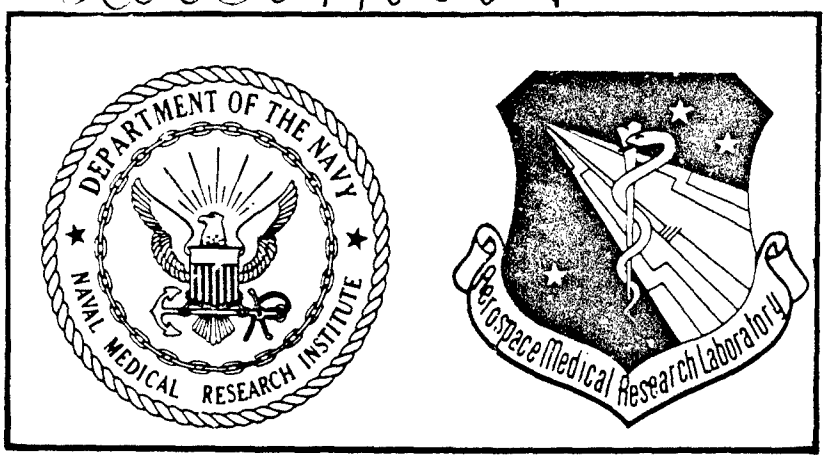

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

BRUCE O. STUART, PhD
Director Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The research program of the Toxic Hazards Research Unit (THRU) for the period of June 1983 through May 1984 is reviewed in this report. Chronic toxicity and oncogenic studies were carried out with hydrazine, JP-4, and JP-8. A series of acute toxicity studies was conducted on a variety of chemicals and chemical agents used by the Army, Air Force, and Navy. Neurotoxicity and subchronic inhalation studies were conducted on several hydraulic fluids and lubricants.

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18. SUBJECT TERMS

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Petroleum Fuels
DEMP
Neurotoxicity
Intraperitoneal
Antimony Thioantimonate
O-ethyl-O'-(2-diisopropylaminoethyl)methylphosphonite
Dimethyl methylphosphonate
Methylcyclohexane
Alveolar Clearance
4-Ipomeanol
Clara Cells
Metabolites
Triarylphosphates

PREFACE

This is the 21st annual report of the Toxic Hazards Research Unit (THRU) and concerns work performed by the Department of Community and Environmental Medicine of the University of California, Irvine on behalf of the Air Force under Contract Number F33615-80-C-0512. This document constitutes the fourth report under the current contract and describes the accomplishments of the THRU from June 1983 through June 1984.

The current contract for operation of the Laboratory was initiated in 1980 under Project 6302, "Occupational and Environmental Toxic Hazards in Air Force Operations", Task 01, "Toxicology of Aerospace Chemicals and Materials", Work Unit Number 63020115. M. K. Pinkerton served as the technical contract monitor for the Air Force Aerospace Medical Research Laboratory.

This is a co-sponsored U. S. Air Force/U. S. Navy research effort. That portion of the work effort sponsored by the U. S. Navy was under the direction of Captain David E. Uddin, MSC, USN, and identified as Navy Task Area Number MF58524001 "Chemical Hazards/Exposure Limits".

J. D. MacEwen, Ph.D., served as Laboratory Director for the THRU of the University of California, Irvine and as co-principal investigator with T. T. Crocker, M.D., Professor, Department of Community and Environmental Medicine. Acknowledgement is made to C. L. Gaworski, C. C. Haun, J. R. Horton, C. E. Johnson, E. R. Kinkead, P. E. Newton, Ph.D., A. K. Roychowdhury, Ph.D., J. L. Monroe, and R. K. Blasingame for their significant contributions and assistance in the preparation of this report. Partial support for this program was provided by the U. S. Naval Medical Research Institute, Toxicology Detachment, Wright-Patterson Air Force Base, Ohio, and the United States Army.

TABLE OF CONTENTS

TABLE OF CONTENTS (CONT'D)

LIST OF FIGURES

LIST OF FIGURES (CONT'D)

LIST OF FIGURES (CONT'D)

LIST OF FIGURES (CONT'D)

LIST OF TABLES

LIST OF TABLES (CONT'D)

LIST OF TABLES (CONT'D)

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SECTION I

INTRODUCTION

The research activity of the Toxic Hazards Research Unit (THRU) is a continuing program independent of contract years, with several studies in progress at the beginning and end of each report period. Experiments that were initiated and completed during the past year and were of sufficient magnitude to merit separate technical reports may only be summarized in this document. Unpublished letter reports are given in detail herein. This year's inhalation research program was conducted on a variety of fuels used for powering aircraft, ships, and rockets and on gas simulants, binary compounds, and a variety of chemical mixtures used as hydraulic fluids. The results or current status of these studies will be discussed in the body of this report. Acute oral and dermal toxicity studies on a variety of materials were also conducted. Delayed neurotoxicity studies were also performed on several compounds.

This document constitutes the 21st annual report of the Toxic Hazards Research Unit, a research team which operates a dedicated inhalation toxicology laboratory to investigate potentially hazardous chemicals and materials of interest to the U. S. Air Force, U. S. Navy, and other governmental agencies. The THRU research team is an interdisciplinary group of University of California, Irvine, toxicologists, chemists, statisticians, and engineers. Support services in pathology, veterinary medicine, and medical technology are provided to the contract by the Air Force.

The research facilities used by the THRU consist of animal exposure chambers and supporting laboratories which have previously been described by MacEwen (1965), Fairchild (1967), and Thomas (1968).

During the first six years of operation, the primary research efforts of the THRU were directed to obtaining information on health hazards of spacecraft flight, and the biological data obtained have been used as criteria for setting continuous exposure limits and for engineering design factors. The primary research efforts have in recent years focused more on problems of aircraft environments, chronic occupational health problems, and the potential oncogenicity of chemicals used in military and

civilian activities. To this end, the current research programs serve the mutual interests of the U. S. Air Force, Navy, Army, and other governmental agencies.

ANNUAL CONFERENCE

As part of its contractual responsibilities, UCI/THRU presents an annual technical conference to disseminate new toxicologic information to the U. S. Air Force and other governmental and industrial scientists. This year's conference was chaired by Anthony A. Thomas, M.D., former Director of the AFAMRL Toxic Hazards Division, now retired from the U. S. government SES. Twenty-four technical platform papers were presented. The theme of this year's conference was Neurotoxicology. The open forum discussions following each session resulted in significant contributions of additional technical information and scientific exchange. The conference, held 15 November through 17 November, 1983 drew 178 participants including speakers.

The welcoming remarks were presented by Billy E. Welch, Ph.D. Chief Scientist, USAF Aerospace Medical Division.

The conference program was submitted to the American Board of Industrial Hygiene and to the University of California, Irvine Continuing Education organization for evaluation. The ABIH awarded 2-1/2 points for recertification and 18 C.E.U.'s were awarded to attending physicians.

The papers presented at the conference were prepared for publication as the Proceedings of the 14th Conference on Environmental Toxicology which is a separate technical report (AFAMRL-TR-83-099).

Our next conference, currently in the development stage, will be held in October 1984 at the Daytonian Hilton Hotel, Dayton, Ohio.

SCIENTIFIC ADVISORY BOARD

As an aid to the overall management of the Toxic Hazards Research Unit, the University of California, Irvine established a panel of scientific advisors in 1972 to review and critique

operation of the THRU laboratory and the scientific merit of research programs conducted under the contract. The Scientific Advisory Board has consisted of 6 to 12 members representing various scientific specialties related to the research programs of the THRU and its broadening projects.

During the current year the Scientific Advisory Board concentrated on a review of the Broadening Program conducted at the UCI campus. Their critique and recommendations resulted in the refocussing of objectives for one program, the reduction of scope in a second, and an increase in manpower to accelerate progress in the third. The Board reviewed the status of the THRU contract which enters its final year beginning September 1984. The Board stressed that the continued importance of the Broadening Programs should be emphasized: "The Broadening Programs have an important beneficial effect on the quality of toxicologic studies performed at the THRU by way of the scientific interactions they foster. They provide clues to toxicologic mechanisms which enable more effective use of data obtained at THRU. A third advantage is that information derived from them aids in future design of efficient experimental protocols."

SECTION II

RESEARCH PROGRAM

Toxicology research conducted by the THRU during the past year was primarily concerned with continuing studies of toxic and tumorigenic effects of inhaled aircraft and rocket fuels. Studies on animals exposed to several repeated bursts of hydrazine vapors equivalent to a cancer causing dose and held for two or more years were completed and the animals were submitted to Air Force pathologists for histopathologic evaluation. A new study on the mechanism of oncogenicity of hydrazine in drinking water was undertaken in collaboration with Dr. Shank and the U.C.I. Toxicology Research Broadening Program. Histologic examinations of animal tissues from several studies on the chronic effects of inhaled aircraft fuels were completed and are described in this report. Subchronic inhalation toxicity studies of dimethyl methylphosphonate, a simulant test gas, were initiated and the exposure phase completed.

Other research activities of the THRU during the past year included a series of acute toxicity tests on O-ethyl-O'-(2-diisopropylaminoethyl)-methylphosphonite (EDMP) and antimony thioantimonate. Hydraulic fluids of various chemical composition were studied for acute toxic effects.

The current status of these ongoing studies is summarized in this report.

THE EVALUATION OF THE ONCOGENIC POTENTIAL OF INHALED HYDRAZINE IN RATS AND HAMSTERS AFTER A SERIES OF WEEKLY ONE-HOUR EXPOSURES

One of the important military uses of hydrazine is as a fuel in standby power systems of aircraft. Maintenance of these systems may result in the occasional accidental brief exposure of aircraft maintenance workers to high concentrations of hydrazine. The intent of this study is to assess the oncogenic risk of several short high concentration exposures to hydrazine. The study design simulated severe intermittent human exposures and utilized the same total dose of hydrazine that had induced pulmonary tumors and nasal polyps in rats and hamsters in previous chronic inhalation exposures.

Background

Hydrazine was shown to be a weak oncogen in rats and hamsters exposed to 5.0 ppm and in rats and mice exposed to 1.0 ppm hydrazine 6 hours/day, 5 days/week for a one-year period (MacEwen and Vernot, 1981). The tumors found from six months to one year after exposure to hydrazine were only seen in the respiratory system where direct contact occurred and were always associated with other lesions produced by the irritative effects of hydrazine on nasal epithelial surfaces. The calculated dose equivalent values or CT (concentration x time) for these exposures was 7500 ppm hours. In order to closely simulate possible accidental exposure of maintenance workers, the present study utilized exposure periods of one hour at the maximum nonlethal concentration for repeated exposure to hydrazine to reach a CT of 7500 ppm hours. The compressed exposure of 7500 ppm hours should be a suitable test for the comparison of short versus long-term exposure at the same CT values. The single weekly one-hour exposures used in the study permit recovery from the acute effects of

hydrazine before subsequent exposure challenges. Rats and hamsters were selected as the test species since a 7500 ppm hour CT of hydrazine has already been demonstrated to produce nasal tumors in each of these species.

Methods

A brief description of the protocol for preliminary studies was presented in the 1980 THRU Annual Report (MacEwen and Vernot, 1980). A complete description of the protocol along with the findings of the Phase I and Phase II portions as well as the Phase III exposure data through May, 1981 was presented in the 1981 THRU Annual Report (MacEwen and Vernot, 1981). Reviews of body weight data and mortality were presented in the 1982 and 1983 THRU Annual Reports (MacEwen and Vernot, 1982, 1983).

Phase I was designed as a range finding study to determine the one-hour LC_{50} values for male and female rats. Preliminary exposures demonstrated, however, that it was impossible to generate sufficiently high vapor concentrations of hydrazine for lethality determinations without aerosol formation. Nevertheless, preliminary exposures indicated the maximum nonlethal level was approximately 750 ppm. The experimental approach for Phase I was, therefore, modified so that 10 male rats, 10 female rats, and 20 male hamsters were exposed to a concentration of 750 ppm hydrazine twice per week for 5 weeks.

The 10 exposures were conducted in a 1 m^3 Rochester Chamber. The chamber concentration of 750 ppm hydrazine was first established and stabilized and then the rats and hamsters in groups of 10 were rapidly inserted into the chamber by means of sliding cage drawers. At the end of one hour the animals were rapidly removed. Four cage drawers were used.

In the absence of a nonexposed control group in this range-finding study, statistical evaluation of the data was not conducted. However, even in the absence of statistical comparisons, the body weight gains of all animal groups exposed to the 750 ppm concentration of hydrazine were adversely affected during the entire exposure regimen. By the 10th exposure, weight loss was seen for male rats and hamsters while female rats showed minimal weight gains. Recovery was seen for all groups by 2 weeks post-exposure. The stress of exposure was reflected in a general unthrifty appearance of the animals, but there was no mortality in any group.

Phase II exposures were conducted in the same manner (sliding cage drawers) and utilized the same chamber as Phase I. Slightly younger animals were used and matched chamber control groups were included. The one-hour exposures were conducted once a week. Ten male rats, ten female rats, and twenty hamsters as well as equivalent numbers of controls were utilized. From these groups 5 male rats, 5 female rats, 10 hamsters, and an equal number of controls were killed after the first one-hour exposure for gross and histologic examination. The remaining animals were killed and examined 24 hours after the final exposure.

The results of Phase II served adequately as a pilot study for Phase III. It demonstrated that repeated weekly one-hour exposures to 750 ppm hydrazine were tolerated by rats and hamsters with no mortality and no significant growth reduction. A total of 900 rodents were used in Phase III to evaluate the oncogenic potential of hydrazine following exposure to the selected concentrations of 750 or 75 ppm. The latter dose was chosen in an attempt to establish a no-effect level. The exposure regimen was the same as that used in Phase II: one hour a week for 10 weeks (total CT values 7500 and 750 ppm hours). The two exposure groups as well as an unexposed control group each consisted of 100 male rats, 100 female rats, and 100 male hamsters.

Results

The body weight graphs and final mortality data for Phase III hamsters were presented in the 1983 THRU Annual Report (MacEwen and Vernot, 1983). Final body weight data for Phase III male and female rats are presented in Figures 1 and 2. These data were examined using a repeated measures multivariate analysis of variance procedure, with the data split into exposure and post-exposure periods. Male and female rat body weight gains of both the low and high level hydrazine exposure groups were significantly ($p < 0.001$) reduced during the exposure period. However, analysis of the postexposure body weight data demonstrated no effects on either male or female body weight gain in either treatment group at the 0.05 level of significance.

Rat mortality data are summarized in Table 1. At 28 months postexposure the male rat mortality reached a level at which it was necessary to terminate those groups in order to have sufficient animals for tissue examination and analysis uncomplicated by postmortem autolysis. There was very little difference in total mortality between control and hydrazine exposed male rat groups. Since female rat survival rate was high, they were held

the full 30 months after exposure initiation at which time tissues were collected from the remaining animals for histologic examination.

Results of the histologic examinations of animals in this study are not available for inclusion in this report. They will be presented in a future annual report.

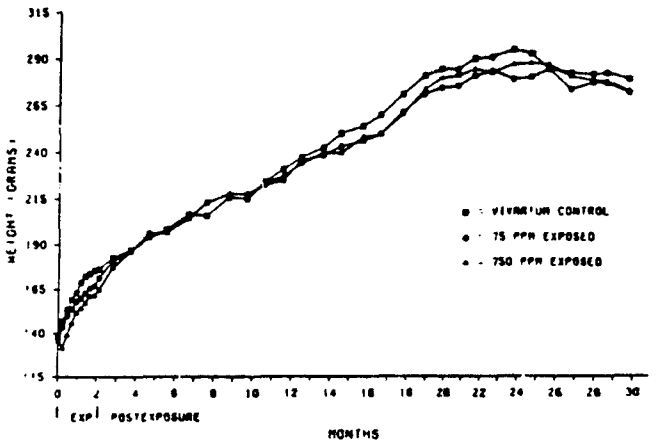

Figure 1. Effect of repeated hydrazine exposure on male rat body weight.

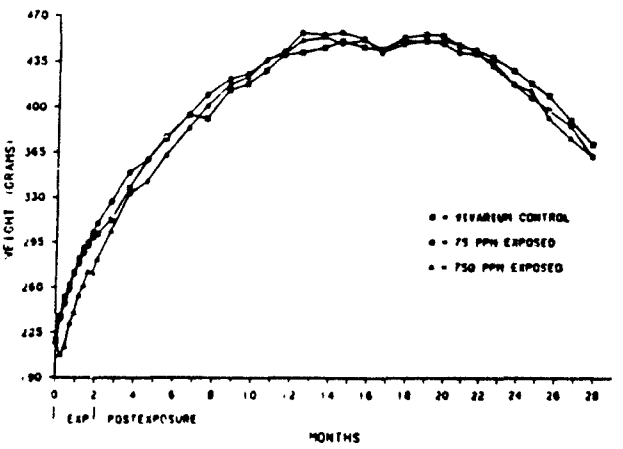

Figure 2. Effect of repeated hydrazine exposure on female rat body weight.

TABLE 1. POSTEXPOSURE MORTALITY OF MALE AND FEMALE FISCHER 344 RATS EXPOSED TO BRIEF HIGH LEVELS OF HYDRAZINE

Sex	Month On Study	Nominal CT (ppm/hours)	Mortality Ratios[a]
Male	28	0	79/93
	28	750	77/93
	28	7500	75/93
Female	30	0	58/93
	30	750	73/93
	30	7500	55/93

[a] Originally 100 animals/group, 7/group sacrificed at 24 months on study.

A TWO-YEAR STUDY ON THE CARCINOGENICITY OF HYDRAZINE ADMINISTERED IN DRINKING WATER TO MALE GOLDEN SYRIAN HAMSTERS

Hydrazine, a strong reducing agent, is widely used in industry and by the military. Under appropriate conditions, repeated hydrazine exposure has been shown to cause an increase in cancer incidence in rats and mice. Hydrazine administration has also been demonstrated to induce the formation of methylated guanines in the livers of test animals. These methylated guanines are thought to be relevant to carcinogenesis (Lawley, 1976). In a recent time-response study Bosan and Shank (1983) showed that after a single dose of hydrazine, O^6-methyl guanine persists in hamster liver DNA much longer than in rat liver. This long persistence of the methylated guanine should greatly increase the carcinogenicity of hydrazine in hamsters provided the liver could be repeatedly exposed. However, in previous studies, hydrazine exposure failed to induce liver cancer (Toth, 1972: MacEwen and Vernot, 1981). Therefore, Dr. Shank (University of California, Irvine) proposed a study in which hamsters would be exposed to high concentrations of hydrazine over prolonged periods of time in order to study the relevance of O^6-methyl guanine in DNA to chemical carcinogenesis.

Dr. Shank hypothesized that continued exposure to hydrazine, and therefore sustained production of O^6-methyl guanine, would induce a carcinogenic response in hamsters. To test this

hypothesis a 2 year study was initiated in which hamsters were provided with drinking water containing high concentrations of hydrazine. At intervals during the exposure hamsters are killed and analyses for O^6-methyl guanine are made in liver, kidney, and lung tissue. A preliminary study conducted by Dr. Shank indicated that hamsters could tolerate drinking water solutions containing up to 0.083% hydrazine sulfate for a 64-day period with minimal effects. During the pilot study hamsters were killed at selected intervals and the livers analyzed for the presence of O^6-methyl guanine and 7-methyl guanine. Both aberrant methylated guanines were present in the livers of the exposed animals. Therefore, a two-year study was designed in collaboration with the Toxic Hazards Research Unit. The animal exposures, necropsies, and tissue preparation for histologic examination are being conducted at the UCI/THRU facilities at Wright-Patterson AFB. Tissue analysis for the methylated guanines and histologic examinations are being done at the Irvine campus.

Methods and Materials

The supplies of hydrazine sulfate and dimethylnitrosamine used in this study were purchased from Aldrich Chemical Co. and Sigma Chemical Co., respectively. The male Golden Syrian hamsters were purchased from the Charles River Breeding Laboratories, Inc., and had a weight range of 50-60 grams on receipt.

Starting in October 1983, three groups of hamsters were supplied hydrazine water solutions as their sole source of drinking water. This will continue for a two-year period. A positive control group receiving dimethylnitrosamine (DMNA) drinking water solution and a negative control group receiving distilled water are also being maintained. Each group consists of 40 animals given the following treatments:

Group 1 - Control, distilled water
Group 2 - Positive control, 10 mg DMNA/liter of distilled water
Group 3 - 170 mg hydrazine sulfate/liter of distilled water
Group 4 - 340 mg hydrazine sulfate/liter of distilled water
Group 5 - 510 mg hydrazine sulfate/liter of distilled water

The hydrazine dose levels are approximately equivalent to 23, 46, and 69 mg hydrazine sulfate/kg of body weight or 5.5, 11, and 17 mg hydrazine/kg of body weight. The drinking water solutions are being prepared fresh twice weekly. Brown glass water bottles are used to prevent photodegradation of the DMNA. Water consumption

was measured twice weekly for two weeks during the first month of exposure. Water consumption will also be measured at 4 additional randomly selected two-week periods during the study. All animals were weighed weekly during the first month of exposure and monthly thereafter. The animals are being maintained in laminar flow enclosures that have been modified to exhaust through an external stack. The hamsters that die during the study will have the livers excised for histologic examination. Tissue preparation will be done at the THRU and the blocks will be sent to the Irvine Campus for histologic evaluation. Three animals from each group will be killed at 6, 12, 18, and 24 months by halothane overdose and the livers, lungs, and kidneys of these animals will be excised, frozen, and shipped to Dr. Shank at Irvine for DNA analysis.

Results

Body weight data are presented in Figure 3. During the first 4 weeks of exposure all groups showed a reduction in growth rate when compared with the distilled water controls. The effect was least noticeable in the DMNA positive controls and was dose related in the hydrazine treated hamsters. With continued treatment the growth effect in the hydrazine treated groups lessened or disappeared, and after 6 months of exposure, the mean body weight of only one group (DMNA) was significantly ($P < 0.05$) less than the negative control.

Water consumption and dose calculations for 2-week periods during the first month of exposure are given in Table 2. There was a dose dependent reduction in water consumption of the hydrazine treated groups ($p < 0.05$) which resulted in a reduction in the average dose per animal from that intended. The hydrazine treatment groups received calculated doses of 14.9, 27.8, or 38.6 mg/kg instead of the projected 23, 46, or 69 mg/kg. Compared with the negative control the hamsters receiving DMNA showed no significant reduction in the amount of drinking water consumed during the 2-week measurement period. Table 3 contains the water consumption and dose calculation data collected during a two-week period in the 5th month of exposure. Only the 170 mg hydrazine group was not different in water consumption from the negative control during this period. All other groups demonstrated reduced water consumption. The doses received by the hamsters were calculated to be 20.6, 29.9, and 36.7 mg hydrazine sulfate/kg, indicating that water consumption and dose levels for the two higher hydrazine groups were constant over the exposure.

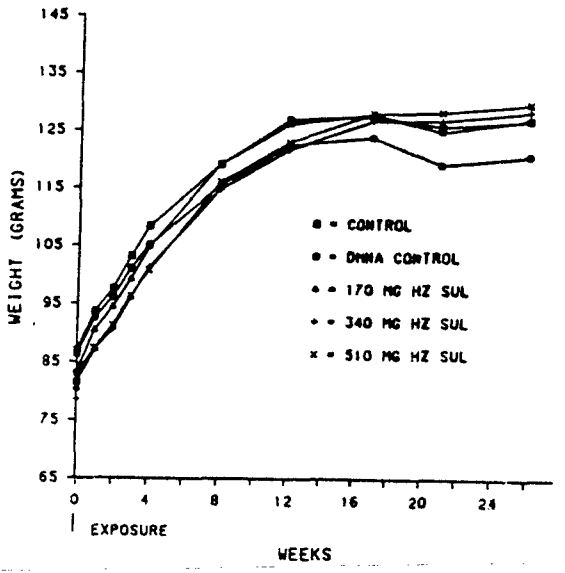

Figure 3. Body weights of male Golden Syrian hamsters receiving various concentrations of hydrazine, DMNA, or no treatment in drinking water.

TABLE 2. WATER CONSUMPTION AND DOSE CALCULATION FOR MALE GOLDEN SYRIAN HAMSTERS DURING TWO WEEKS OF THE FIRST MONTH OF HYDRAZINE EXPOSURE

Period	Control	Hydrazine Sulfate			
		10 mg DMNA	170 mg/L	340 mg/L	510 mg/L
Water consumption ml/animal/day)	9.2	9.8	8.7	7.9	7.3
Dose (mg/animal/day)	---	0.098	1.48	2.70	3.71
Animal body wt. (g) on 11/8/83		102	99	97	96
Dose (mg/kg/day)		0.96	14.9	27.8	38.6

TABLE 3. WATER CONSUMPTION AND DOSE CALCULATIONS FOR MALE GOLDEN SYRIAN HAMSTERS DURING TWO WEEKS OF THE FIFTH MONTH OF HYDRAZINE EXPOSURE

Period	Control	Hydrazine Sulfate			
		10 mg DMNA	170 mg/L	340 mg/L	510 mg/L
Water consumption (ml/animal/day)	14.8	11.8	15.2	11.2	9.2
Dose (mg/animal/day)	--	0.118	2.58	3.79	4.70
Animal body wt. (g) on 3/18/84		119	125	127	128
Dose (mg/kg/day)		0.99	20.6	29.9	36.7

Mortality data are presented in Table 4. The only group that is significantly different from the others is the DMNA group. The mortality ratio for this group is much higher than would be expected. A review of the gross necropsy records revealed that all hamsters that died in the DMNA treatment group had abnormal livers. The livers were characterized as yellow-green in color, brittle, and nodular in appearance. There has not been significant mortality in any of the hydrazine treatment groups. Because of the high mortality appearing in the DMNA treatment group, exposure to DMNA was halted in May 1984 after approximately seven months of exposure. The hamsters remaining in the group are receiving distilled water.

TABLE 4. MORTALITY RATIOS[a] OF MALE GOLDEN SYRIAN HAMSTERS AFTER TREATMENT WITH HYDRAZINE OR DMNA IN DRINKING WATER

Species	Treatment Group				
	DMNA	Hydrazine Sulfate			
	Control	10 mg/L	170 mg/L	340 mg/L	510 mg/L
Hamster	1/37	9/37	0/37	1/37	1/37

[a] Mortality ratios have been adjusted for the six-month interim sacrifice.

As scheduled in the protocol for the study a 6-month interim sacrifice was conducted in April 1984. Tissues collected were sent to Dr. R. Shank at the Irvine Campus for analysis. Progress of this study will be updated in future annual reports.

TWELVE-MONTH CHRONIC INHALATION EXPOSURES TO METHYLCYCLOHEXANE

The exposure portion of this study began in August 1978 and continued for one year after which 20 mice, 10 rats, and 10 hamsters from each group were necropsied to assess chronic toxicity effects in primary tissues. The remaining rodents were held for an additional year of observation and the dogs will continue to be held through July 1984. Each exposure and control group of animals consisted of 65 male and 65 female rats, 200 female mice, 100 male hamsters, and 8 dogs equally divided by sex. The numbers of rodents used were selected to provide a statistically valid number of each sex and species which had reached the required age for tumor induction allowing for natural attrition.

The experimental protocol, methodology of inhalation exposure, and clinical data obtained during the 12-month exposure phase were described in previous annual reports (MacEwen and Vernot, 1980, 1981). This report updates the histopathologic evaluation of the exposed rodents necropsied for this purpose at the end of the 12-month intermittent exposure phase and includes the histopathologic evaluation of the animals killed at the end of the 12-month postexposure observation period.

Background

Methylcyclohexane (MCH) is a constituent of jet aircraft fuel JP-9. This fuel is a mixture of three primary ingredients, JP-10, RJ-5, and MCH. JP-10 and RJ-5 are high density hydrocarbons yielding a greater BTU output than conventional aircraft fuels. They also have high viscosities which cause pumping and flow problems at low temperature that are corrected by the addition of MCH to the mixture.

In 1976, the American Conference of Governmental Industrial Hygienists lowered the threshold limit value (TLV) for MCH from 500 ppm to 400 ppm or 1600 mg/m^3. The recommended short-term

exposure limit (STEL) is 500 ppm or 2000 mg/m^3. These values are based on analogy to the toxicity of heptane and are identical to the TLV and STEL of heptane.

The scarcity of chronic exposure data for animals with the consequent use of analogy to other solvents makes the setting of human exposure limits unreliable. Prolonged exposures to methylbutylketone and n-hexane have been shown to cause peripheral polyneuropathy in man (Billmaier et al., 1974; Allen et al., 1975). A TLV of 500 ppm had been set for n-hexane based solely on acute toxicity data and comparison with other petroleum solvents such as pentane. Reports of neuropathy in workers exposed to hexane resulted in the lowering of the American Conference of Governmental Industrial Hygienists TLV to 100 ppm in 1977.

These studies were undertaken to obtain the data needed to assess the safety margin of current exposure limits for methylcyclohexane. The design of the study also provides for the detection of oncogenic potential of methylcyclohexane.

Animal exposure concentrations of MCH for this study were selected on the basis of the current TLV (400 ppm) and the maximum tolerated level for repeated exposures which appeared to be 2000 ppm.

Micropathologic tissue changes seen in the rodents up to and including the planned sacrifice at the end of the 12-month intermittent exposure to MCH are listed in Tables 5-7 for rats, mice, and hamsters, respectively.

Since only a small number of lesions were observed in these animals, both tumors and nontumorous lesions are tabulated together. Only one tumor was noted in any female rat - a benign endometrial stromal polyp found in one rat from the 400 ppm MCH exposure group. The other lesions noted were of a nonsignificant nature.

The tumors seen in the male rats are commonly found in this strain, although the onset of interstitial cell tumors of the testes may be advanced. There appeared to be a slight increase in dilatation of renal tubules in the 2000 ppm MCH exposed male rats but no other indication of kidney injury was seen at this time.

TABLE 5. TISSUE CHANGES SEEN IN MALE AND FEMALE FISCHER 344 RATS AT THE END OF 12-MONTH INTERMITTENT EXPOSURE TO INHALED METHYLCYCLOHEXANE

	Unexposed Controls	400 ppm Exposed	2000 ppm Exposed
Male Rats:			
Pituitary Adenoma	2	0	1
Testicular Tumor	0	5[a]	2
Adrenal Pheochromocytoma	1	1	0
Bile Duct Hyperplasia	1	2	0
Renal Tubular Dilatation	1	2	4
Lungs:			
Lymphocytic Infiltrates	2	0	1
Arterial Mineralization	2	1	0
Myocardial Fibrosis	2	3	0
Number of Animals Examined	11	10	11
Female Rats:			
Ovarian Cyst	0	4	2
Lungs:			
Lymphocytic Infiltrates	6	0	3
Arterial Mineralization	1	1	1
Endometrial Stromal Polyp	0	1	0
Number of Animals Examined	11	10	10

[a] Statistically different from control incidence at $p < 0.05$.

Only one tumor, a benign tumor of an adrenal gland, was seen in all hamsters examined. This tumor was seen in the 2000 ppm MCH exposure group. The other incidental lesions seen were fairly uniform in all groups and not related to MCH exposure.

The results of the examination of tissue from the animals that died during the postexposure observation period or were killed at the study termination are listed in Tables 8 through 14. The tables of non-neoplastic lesions of the three species have been abbreviated to exclude lesions of very low incidence. In male rats, the major target organ was the kidney where several lesions demonstrated clear exposure and dose relationships. Virtually all of the male rats had lesions consistent with progressive renal nephropathy, common in older rats. In the rats exposed to the higher level, there was a statistically

significant increase in the occurrence of medullary mineralization and epithelial hyperplasia of the renal papilla. Interstitial cell tumors of the testes, seen at study termination, appear to be equally distributed between the test and control groups and not related to exposure. No dose related lesions were noted in the exposed female rats when compared to the control group.

TABLE 6. TISSUE CHANGES SEEN IN FEMALE C57BL/6 MICE AT THE END OF 12-MONTH INTERMITTENT EXPOSURE TO INHALED METHYLCYCLOHEXANE

	Unexposed Controls	400 ppm Exposed	2000 ppm Exposed
Lung:			
Lymphoid Hyperplasia	1	4	3
Alveolar Bronchiolar Adenoma	1	0	0
Liver:			
Fatty change	10	7	5
Hyperplasia	3	4	1
Malignant Lymphoma	3	4	4
Pituitary Adenoma	3	0	0
Uterine Cysts	18	16	14
Kidney Hyperplasia	5	4	2
Number of Animals Examined	29	35	39

TABLE 7. TISSUE CHANGES SEEN IN MALE GOLDEN SYRIAN HAMSTERS AT THE END OF 12-MONTH INTERMITTENT EXPOSURE TO INHALED METHYLCYCLOHEXANE

	Unexposed Controls	400 ppm Exposed	2000 ppm Exposed
Kidney Mineralization	5	4	1
Renal Tubular Dilatation	7	1	3
Pituitary Fatty Change	2	4	3
Adrenal Adenoma	0	0	1
Number of Animals Examined	24	20	17

No significant non-neoplastic lesions were found in female mice or male hamsters when compared to their respective control groups. Lesions noted were those commonly seen in older mice and hamsters.

TABLE 8. SELECTED NON-NEOPLASTIC LESIONS[a] SEEN IN RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Unexposed Controls	400 ppm Exposed	2000 ppm Exposed
Males			
Liver			
Bile Duct Hyperplasia	32/53	22/55	19/52
Necrosis	2/53	0/55	1/52
Circulatory System			
Myocardial Fibrosis	11/53	3/55	14/52
Pulmonary Artery Mineralization	6/53	3/55	0/52
Kidney			
Medullary Mineralization	1/53	2/55	19/52[b]
Nephropathy	49/54	52/55	52/52
Papillary Hyperplasia	1/53	1/55	23/52[b]
Tubular Degeneration	1/53	0/55	2/52
Testes			
Atrophy	4/53	2/55	1/52
Lungs			
Adenomatosis	1/53	2/55	0/52
Females			
Liver			
Bile Duct Hyperplasia	5/52	2/50	3/54
Necrosis	4/52	0/50	1/54
Circulatory System			
Myocardial Fibrosis	1/52	3/51	4/53
Pulmonary Artery Mineralization	6/52	2/51	3/54
Kidney			
Medullary Mineralization	4/52	0/51	1/54
Nephropathy	15/52	7/51	15/54
Reproductive			
Ovarian Cysts	6/50	2/51	3/52
Uterine Dilatation	5/52	9/51	4/52
Mammary Gland			
Cystic Hyperplasia	10/47	17/53	14/48
Lungs			
Adenomatosis	2/52	0/51	1/54

[a] Number of lesions observed/number of animals examined.
[b] Statistically different from control incidence at $p < 0.01$.

TABLE 9. NEOPLASTIC LESIONS[a] SEEN IN MALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Skin/Subcutaneous			
Keratoacanthoma	0/51	1/55	3/52
Fibroma	3/53	4/55	0/52
Fibroadenoma	0/53	1/55	0/52
Osteosarcoma	1/53	0/55	0/52
Basal Cell Tumor	0/53	1/55	1/52
Mammary Gland Fibroadenoma	0/46	0/47	2/52
Myxoma	1/53	0/55	0/52
Lungs			
Squamous Cell Carcinoma	0/54	1/55	0/52
Nasal			
Squamous Cell Carcinoma	1/53	0/55	0/52
Liver			
Mononuclear Cell Leukemia	0/53	0/55	0/52
Pituitary			
Adenoma	17/51	11/54	16/48
Carcinoma	2/51	1/54	0/48
Neoplasm	1/51	0/54	0/48
Thyroid			
Adenoma	4/52	5/54	5/51
Carcinoma	0/52	1/54	2/51
Kidney			
Renal Cell Adenoma	0/54	0/55	1/52
Renal Cell Carcinoma	0/54	1/55	0/52
Adrenals			
Adenoma	1/54	1/55	5/52
Carcinoma	0/54	1/55	0/52
Pheochromocytoma	3/54	0/55	2/52
Stomach			
Leiomyoma	0/53	0/54	1/52
Pancreas			
Islet Cell Adenoma	1/53	1/54	1/51
Testis			
Interstitial Cell Tumor	49/54	49/55	50/52
Zymbals Gland			
Squamous Cell Carcinoma	0/54	0/55	1/52
Preputial Gland			
Adenocarcinoma	0/54	0/55	1/52
Parathyroid			
Adenoma	1/54	0/55	0/52
Multiple Organ			
Mesothelioma	1/54	1/55	1/52
Malignant Lymphoma	1/54	2/55	0/52
Bronchial Mucous Gland			
Adenoma	0/54	1/55	0/52
Histiocytic Leukemia	0/54	3/55	2/52

[a] Number of lesions observed/number of animals examined.

TABLE 10. NEOPLASTIC LESIONS[a] SEEN IN FEMALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Skin			
Keratoacanthoma	0/49	0/54	2/51
Fibroma	1/52	0/51	3/51
Trichoepithelioma	1/52	0/51	0/51
Fibroadenoma	1/52	3/51	3/51
Adenoma	1/52	0/51	0/51
Sarcoma, Undifferentiated	0/52	1/51	0/51
Sarcoma	0/52	1/51	0/51
Mammary Gland Fibroadenoma	0/47	4/50	6/48
Lungs			
Alveolar/Bronchiolar Carcinoma	0/52	1/54	0/54
Osteosarcoma	0/52	0/54	1/54
Sarcoma	0/52	1/54	0/54
Pituitary			
Adenoma	11/50	16/50	17/54
Carcinoma	3/50	4/50	5/54
Thyroid			
Adenoma	1/52	1/52	2/51
Carcinoma	2/52	3/52	1/51
Parathyroid			
Adenoma	0/31	1/40	0/35
Mediastinal Lymph Node			
C-Cell Carcinoma	0/52	1/54	0/54
Adrenals			
Adenoma	0/52	1/53	1/54
Adenocarcinoma	1/52	0/53	0/54
Pancreas			
Adenocarcinoma	1/51	0/54	0/50
Uterus			
Endometrial Stromal Polyp	7/52	4/54	0/52
Adenocarcinoma	3/52	0/54	0/52
Leiomyosarcoma	0/52	1/54	0/52
Urinary Bladder			
Adenocarcinoma	1/45	0/53	0/51
Brain			
Astrocytoma	0/52	1/54	0/53
Clitoral Gland			
Adenoma	2/52	0/54	0/53
Abdominal Cavity			
Lipoma	1/52	0/54	1/53
Adenocarcinoma	1/52	0/54	3/53
Mesothelioma	0/52	1/54	0/53
Myxosarcoma	0/52	0/54	1/53
Circulatory System			
Histiocytic Leukemia	2/52	2/54	5/53
Malignant Lymphoma	1/52	0/54	0/53

[a] Number of lesions observed/number of animals examined.

TABLE 11. SELECTED NON-NEOPLASTIC LESIONS[a] SEEN IN FEMALE MICE HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Lungs			
Alveolar Crystals	24/170	8/158	6/155
Alveolar Macrophages	31/170	17/158	9/155
Perivascular Cuffing	20/170	25/158	19/155
Lymphoid Hyperplasia	20/170	23/158	25/155
Spleen			
Hematopoiesis	22/164	34/150	23/154
Liver			
Fatty Change	36/171	14/159	16/155
Hematopoiesis	20/171	20/159	24/155
Duodenum			
Mesentery Strangulation	19/167	7/150	10/151
Kidney			
Hydronephrosis	10/171	13/159	7/155
Perivascular Cuffing	13/171	14/159	8/155
Uterus			
Multiple Cysts	10/164	22/158	23/152
Endometrial Dilatation	30/164	38/158	29/152
Ovaries			
Cysts	19/149	20/155	24/135
Hemorrhagic Cysts	12/149	14/155	12/135
Mammary Gland			
Cystic Hyperplasia	26/145	10/118	12/119
Thyroid Gland			
Papillary Hyperplasia	79/164	39/151	44/145

[a] Number of lesions observed/number of animals examined.

TABLE 12. NEOPLASTIC LESIONS[a] SEEN IN FEMALE MICE HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Skin/Subcutaneous			
Keratoacanthoma	0/167	0/154	1/150
Fibroma	1/167	0/154	0/150
Lung			
Alveolar/Bronchiolar Adenoma	4/170	6/158	0/155
Alveolar/Bronchiolar Carcinoma	3/170	1/158	3/155
Lymph Node			
Hemangiosarcoma	1/168	0/150	0/146
Heart			
Carcinoma	1/170	0/158	0/155
Liver			
Hepatocellular Adenoma	0/171	1/152	0/152
Hemangiosarcoma	0/171	0/152	1/152
Duodenum			
Papilloma	1/167	0/150	0/151
Papillary Adenoma	1/167	1/150	0/151
Uterus			
Neoplasm	1/164	0/158	1/152
Leiomyosarcoma	0/164	1/158	1/152
Ovaries			
Adenoma	1/149	0/155	0/135
Tubular Adenoma	1/149	1/155	4/135
Pituitary			
Adenoma	72/142	40/142	44/118
Carcinoma	4/142	0/142	0/118
Adenocarcinoma	5/142	0/142	2/118
Adrenal			
Adenoma	0/170	0/158	1/149
Thyroid			
Adenoma	0/164	1/151	0/145
Follicular-Cell Adenoma	2/164	1/151	1/145
Lacrimal Gland			
Adenoma	0/171	1/162	0/155
Bone			
Osteosarcoma	0/162	1/159	0/151
Circulatory System			
Malignant Lymphoma	45/171	44/162	56/155
Leukemia	0/171	0/162	1/155

[a] Number of lesions observed/number of animals examined.

TABLE 13. SELECTED NON-NEOPLASTIC LESIONS[a] SEEN IN MALE HAMSTERS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Kidney			
Cortical Fibrosis	4/75	12/76	10/81
Mineralization, Collecting Tubules	26/75	19/76	24/81
Mineralization, Convoluted Tubules	14/75	10/76	8/81
Dilatation, Convoluted Tubules	17/75	16/76	17/81
Mineralization, Renal Pelvis	10/75	0/76	6/81
Testis			
Atrophy	4/76	3/76	2/81
Aspermatogenesis	3/76	4/76	5/81
Adrenal Gland			
Cortical Hyperplasia	35/75	30/76	29/80

[a] Number of lesions observed/number of animals examined.

Neoplastic changes seen in all three species were, for the most part, common for the species. Statistical analysis of the data failed to indicate any significant increase in tumor formation in the MCH exposed animals when compared to their respective controls.

Discussion

The major toxic effect of exposure to methylcyclohexane vapors has been on the male rat kidney. Exposure related renal change has not been found in female rats, female mice, or male hamsters. Immediately following the 12-month exposure period, renal tubular dilatation was seen in 36% of male rats exposed to the high level, 20% of those exposed to the low level, and only

9% of the controls. Associated kidney lesions during or after the one year postexposure observation period were seen only in male rats exposed to 2000 ppm MCH.

TABLE 14. NEOPLASTIC LESIONS[a] SEEN IN MALE HAMSTERS HELD FOR POSTEXPOSURE OBSERVATION AFTER 12-MONTH INTERMITTENT INHALATION EXPOSURE TO METHYLCYCLOHEXANE

	Control	400 ppm	2000 ppm
Skin/Subcutaneous			
Fibroma	0/74	0/76	1/80
Trachea			
Adenoma	0/72	0/75	2/79
Spleen			
Hemangiosarcoma	0/71	1/75	1/78
Lymph Node			
Neoplasm	1/73	0/74	0/78
Liver			
Carcinoma	0/74	0/77	1/80
Islet-Cell Carcinoma, metastatic	0/74	1/77	0/80
Hepatocellular Carcinoma	0/74	1/77	0/80
Hemangiosarcoma	0/74	1/77	1/80
Angioma	0/74	1/77	0/80
Pancreas			
Islet-Cell Carcinoma	0/63	1/72	1/75
Duodenum			
Undifferentiated Sarcoma	0/71	0/76	1/79
Kidneys			
Renal-Cell Carcinoma	0/75	1/76	0/81
Adrenal Gland			
Carcinoma	2/75	3/76	7/80
Adenoma	18/75	21/76	12/80
Adenocarcinoma	1/75	0/76	0/80
Pheochromocytoma	0/75	1/76	0/80
Thyroid			
C-Cell Adenoma	0/71	0/67	1/71
C-Cell Carcinoma	2/71	1/67	0/71
Parathyroid			
Adenoma	1/45	0/43	1/47
Multiple Organs			
Carcinoma	0/76	0/77	1/82
Sarcoma	0/76	0/77	1/82
Malignant Lymphoma	2/76	5/77	4/82
Myelogenous Leukemia	0/76	1/77	0/82

[a] Number of lesions observed/number of animals examined.

Under the conditions of this study, methylcyclohexane exhibited no carcinogenic activity. The results indicate no substantial increase in tumor formation in exposed animals when compared to controls. Tumors noted in all groups were common to the species.

Semiannual clinical determinations performed on dogs for 5 years following exposure to 400 and 2000 ppm show no indication of renal effects. Dogs are scheduled to be killed July 1984. The results of the microscopic examination of dog tissue will be discussed in a future report.

The results of this study support the selection and safety of the current TLV of 400 ppm for methylcyclohexane.

NINETY-DAY CONTINUOUS INHALATION EXPOSURE TO JP-8 JET FUEL

As part of a series of studies investigating the toxic hazards associated with inhalation of hydrocarbon fuels, the THRU conducted a 90-day continuous inhalation exposure with the jet fuel designated JP-8. The results of this study will be useful in a comparative evaluation of the hydrocarbon fuels used by the Air Force.

Tests with JP-4 and JP-5, petroleum fuels similar to JP-8, have shown histopathologic changes in the kidneys of exposed male rats. JP-8 was also expected to induce these effects; therefore special emphasis was placed on the evaluation of renal toxicity.

Since the last annual report the animals have completed one year of postexposure observation. Sacrifices at 2 and 9 months postexposure were conducted in May and December 1983 as scheduled in the study protocol. The results of clinical tests made at that time are presented in this report.

A detailed discussion of the experimental procedures and tests conducted during the study along with a description of the JP-8 generation and analysis methods was presented in the last annual report (MacEwen and Vernot, 1983).

The exposure of rats and mice to JP-8 vapors began in December 1982 and terminated in March 1983. Two exposure concentrations were used, 500 mg/m^3 and 1000 mg/m^3. Sham exposed controls were also maintained in the Thomas Dome inhalation chambers used

for the study. Each chamber housed 95 male and 75 female Fischer 344 rats and 100 male and 100 female C57BL/6 mice. Following completion of the 90-day exposure, 15 rats and 25 mice of each sex were killed and tissue collected for examination.

Additional samplings were conducted at 2 weeks postexposure (10 male rats/group), 2 months postexposure (10 male rats/group), and 9 months postexposure (10 rats and mice of each sex/group). All remaining animals will be necropsied during the 24th month of the study (December, 1984).

At each of the scheduled tissue sampling times, blood and urine samples were also collected from the rats for examination. Rat organ weights were measured at exposure termination and 9 months postexposure. Organ weights will also be measured at study termination. Body weights of the rats and mice were routinely measured during the course of the exposure and are also being followed postexposure.

Results

The body weights of male rats are shown in Figure 4. At exposure termination both groups of male rats exposed to JP-8 vapors demonstrated significantly ($p < 0.05$) reduced weight gain compared to the sham exposed control group. This trend continued for the first six or seven months postexposure. However, at one year postexposure only the low level exposure group exhibited a weight difference from the control group that was significant at $p < 0.05$. Female rats exposed to 1000 mg/m^3 weighed significantly ($p < 0.05$) less than controls during the latter half of the exposure (Figure 5). Postexposure body weights of JP-8 exposed female rats were not significantly different from the controls.

Mortality incidence for the rodents exposed to JP-8 vapors is presented in Table 15. Mortality has been low in all groups of male and female rats with little difference between control and exposed groups. Overall mortality was greater in both sexes of mice exposed to JP-8 compared to respective control groups. This was particularly evident in the male mice where the deaths in each exposure group were twice as great as in the control group. Moribund sacrifice due to lesions resulting from chronic dermatitis has accounted for 12, 22, and 25 of the deaths of male mice in the control, low concentration and high concentration

groups, respectively. While the incidence was not as great, moribund sacrifice related to chronic dermatitis was also the major cause of death in female mice.

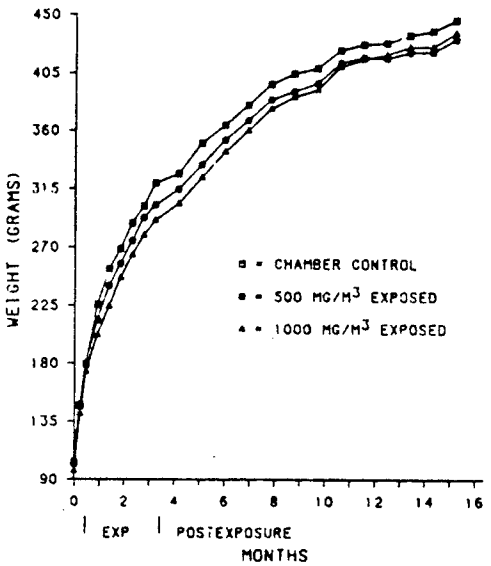

Figure 4. Body weights of male rats exposed continuously for 90 days to JP-8.

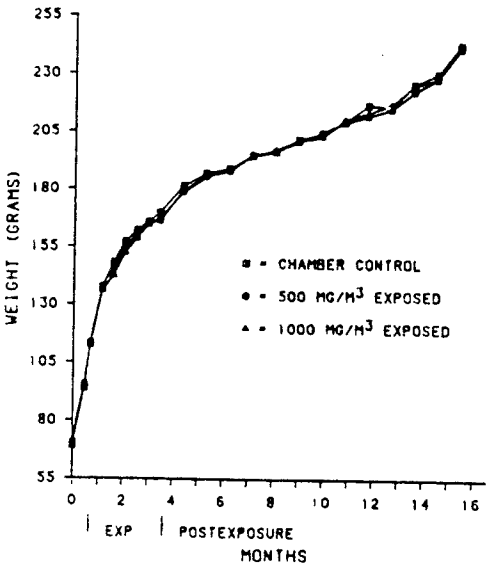

Figure 5. Body weights of female rats exposed continuously for 90 days to JP-8.

TABLE 15. MORTALITY IN RODENTS EXPOSED CONTINUOUSLY FOR 90 DAYS TO JP-8 VAPOR[a,b]

	Control	500 mg/m^3	1000 mg/m^3
C57BL/6 Mice, Male	17/65	30/65	36/64
C57BL/6 Mice, Female	4/65	14/65	11/64
Fischer 344 Rats, Male	6/50	3/49	3/50
Fischer 344 Rats, Female	0/50	0/50	1/50

[a] Denominator adjusted to exclude animals from scheduled sacrifices, missing or dying accidentally. Original N values = 100 mice/sex, 85 rats/sex.

[b] One year postexposure.

Results of blood tests conducted on the male rats sacrificed at 2 months postexposure are shown in Table 16. Slight but statistically significant reductions in RBC counts, hematocrit and hemoglobin were indicated in male rats exposed to 1000 mg/m^3. BUN and creatinine levels in exposed male rats were not different from controls.

Examination of blood collected from rats 9 months postexposure indicated a number of the hematology and clinical chemistry values of male rats exposed to 1000 mg/m^3 to be different from controls (Table 17). All values were within normal species variation, however. Reduced red blood cell counts, hematocrit and hemoglobin values in male rats exposed to JP-8 vapors at 1000 mg/m^3 were again noted. Female rat blood values are shown in Table 18. White blood cell counts for all groups of female rats, control and exposed, were lower than normal for the Fischer 344 strain of rat. Values for the other blood parameters measured were within normal variation and fail to suggest any marked exposure related effects.

Urinalysis at 2 months postexposure failed to indicate any differences between JP-8 exposed and control rat urine. Instrument malfunction prevented the determination of urine osmolality at 2 months postexposure. Epithelial cells noted with increased frequency in the urine of exposed male rats at exposure termination and 2 weeks postexposure were not seen in the urine collected 2 months postexposure.

TABLE 16. HEMATOLOGY AND SERUM CHEMISTRY VALUES[a] OF MALE RATS 2 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO JP-8 VAPOR

	Control	500 mg/m^3	1000 mg/m^3
RBC ($x10^6$ cells/mm^3)	7.72 ± 0.12[d]	7.49 ± 0.23	7.06 ± 0.11[b]
WBC ($x10^3$ cells/mm^3)	4.07 ± 0.3[d]	7.6 ± 0.3[b]	4.7 ± 0.2
HCT (%)	38.9 ± 0.7[d]	37.4 ± 1.1	35.7 ± 0.5[c]
HGB (g/dl)	14.8 ± 0.3[d]	15.2 ± 0.2	13.6 ± 0.2[b]
MCV (um^3)	50.4 ± 0.3[d]	50.0 ± 0.2	50.6 ± 0.2
MCH (pg)	19.01 ± 0.2[d]	20.4 ± 0.5[c]	19.3 ± 0.2
MCHC (mg/dl)	37.9 ± 0.4[d]	40.7 ± 1.0[c]	38.1 ± 0.5
BUN (mg/dl)	18.6 ± 0.8	17.3 ± 0.6	19.1 ± 0.9
Creatinine (mg/dl)	0.51 ± 0.02	0.53 ± 0.02	0.53 ± 0.02

[a] Mean ± SE, (N = 10).
[b] Statistically different from control value, $p < 0.01$.
[c] Statistically different from control value, $p < 0.05$.
[d] N = 8.

The urine osmolality values of all groups of male rats examined 9 months postexposure were generally lower than the values obtained at two weeks postexposure (Table 19). There was no significant difference in urine osmolality among the three groups examined 9 months postexposure. Microscopic examinations of the urine collected 9 months postexposure revealed small numbers of white blood cells, red blood cells, and epithelial cells in the urine of all three groups. White blood cells and red blood cells had not previously been noted in the urine collected from animals in this study, while epithelial cells were seen in exposed male rat urine examined at exposure termination and 2 weeks postexposure.

Organ weights measured at the 9-month postexposure sacrifice are shown in Table 20. A slight but significant increase in kidney to body weight ratios for the 1000 mg/m^3 male rat group was seen when compared to the control value. No other significant organ weight differences were noted. Examination of organ weights at exposure termination had shown increased liver weight in male rats exposed to 1000 mg/m^3 and in female rats exposed to

either concentration. Substantial elevations in kidney weights were also noted at that time in male rats exposed to either concentration of JP-8 vapors.

TABLE 17. HEMATOLOGY AND SERUM CHEMISTRY VALUES[a] OF MALE RATS 9 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO JP-8 VAPOR

	Control	500 mg/m^3	1000 mg/m^3
WBC (x10^3 cells/mm^3)	5.6 ± 0.4	6.2 ± 0.4	5.0 ± 0.4[e]
RBC (x10^6 cells/mm^3)	8.47 ± 0.13	8.16 ± 0.07	7.73 ± 0.05[e]
HGB (g/dl)	15.6 ± 0.2	15.1 ± 0.2	14.8 ± 0.1[ce]
HCT (%)	41.7 ± 0.6	40.1 ± 0.4	37.5 ± 0.3[be]
MCV (um^3)	49.2 ± 0.2	49.1 ± 0.3	48.6 ± 0.1[de]
MCH (pg)	18.4 ± 0.1	18.5 ± 0.2	19.2 ± 0.1[ce]
MCHC (g/dl)	37.4 ± 0.2	37.7 ± 0.5	39.5 ± 0.1[be]
Glucose (mg/dl)	187 ± 7	177 ± 7	133 ± 8[b]
Tot. Prot. (g/dl)	7.48 ± 0.12	7.43 ± 0.08	7.13 ± 0.07[d]
Albumin (g/dl)	1.17 ± 0.01	1.03 ± 0.03[c]	0.90 ± 0.03[b]
Globulin (g/dl)	6.31 ± 0.12	6.40 ± 0.10	6.23 ± 0.09
A/G Ratio	0.19 ± 0.003	0.16 ± 0.01[d]	0.15 ± 0.01[b]
BUN (mg/dl)	19.3 ± 0.5	18.1 ± 0.4	18.6 ± 0.7
Creatinine (mg/dl)	0.6 ± 0.01	0.6 ± 0.03	0.5 ± 0.03
Calcium (mg/dl)	11.2 ± 0.1	11.2 ± 0.1	10.6 ± 0.1[b]
SGOT (IU/L)	108 ± 15	86 ± 3	108 ± 3
SGPT (IU/L)	72 ± 7	56 ± 2[d]	55 ± 2[d]
Alk. Phos. (IU/L)	117 ± 6	98 ± 5	89 ± 5[c]
Bilirubin (mg/dl)	0.3 ± 0.02	0.3 ± 0.02	0.3 ± 0.01

[a] Values expressed as mean ± SE, (N = 10).
[b] Statistically different from control at $p < 0.001$.
[c] Statistically different from control at $p < 0.01$.
[d] Statistically different from control at $p < 0.05$.
[e] N = 9.

TABLE 18. HEMATOLOGY AND SERUM CHEMISTRY VALUES[a] OF FEMALE RATS 9 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO JP-8 VAPOR

	Control (N = 9, 10)	500 mg/m^3 (N = 8, 9)	1000 mg/m^3 (N = 6-10)
WBC (x10^3 cells/mm^3)	3.0 ± 0.2	3.2 ± 0.1	4.3 ± 0.3[b]
RBC (x10^6 cells/mm^3)	7.89 ± 0.07	7.77 ± 0.15	7.86 ± 0.23
HGB (g/dl)	15.0 ± 0.1	15.1 ± 0.2	16.3 ± 0.4[c]
HCT (%)	43.9 ± 0.3	42.4 ± 0.9	42.1 ± 1.3
MCV (um^3)	55.6 ± 0.2	54.5 ± 0.3[c]	53.5 ± 0.2[b]
MCH (pg)	19.0 ± 0.1	19.4 ± 0.3	20.8 ± 0.2[b]
MCHC (g/dl)	34.2 ± 0.1	35.7 ± 0.7	38.8 ± 0.4[b]
Glucose (mg/dl)	137 ± 5	123 ± 8	136 ± 5
Tot. Prot. (g/dl)	8.00 ± 0.21	8.25 ± 0.09	7.80 ± 0.16
Albumin (g/dl)	1.18 ± 0.01	1.22 ± 0.01	1.18 ± 0.02
Globulin (g/dl)	6.71 ± 0.17	7.03 ± 0.08	6.63 ± 0.15
A/G Ratio	0.18 ± 0.004	0.17 ± 0.002	0.18 ± 0.002
BUN (mg/dl)	18.7 ± 1.0	16.3 ± 0.9	18.8 ± 0.8
Creatinine (mg/dl)	0.5 ± 0.02	0.6 ± 0.03	0.6 ± 0.02
Calcium (mg/dl)	10.7 ± 0.1	10.7 ± 0.1	10.6 ± 0.2
SGOT (IU/L)	83 ± 10	104 ± 10	76.0 ± 3
SGPT (IU/L)	40 ± 4	45 ± 3	47 ± 3
Alk. Phos. (IU/L)	56 ± 4	74 ± 15	57 ± 4
Bilirubin (mg/dl)	0.2 ± 0.02	0.2 ± 0.03	0.1 ± 0.02[d]

[a] Values expressed as mean ± SE.
[b] Statistically different from control at $p < 0.001$.
[c] Statistically different from control at $p < 0.01$.
[d] Statistically different from control at $p < 0.05$.

TABLE 19. EFFECT OF 90-DAY CONTINUOUS INHALATION OF JP-8 VAPORS ON RAT URINE OSMOLALITY (mOsm)[a]

	Control	500 mg/m^3	1000 mg/m^3
Preexposure	1448 ± 89	1462 ± 83	1413 ± 113
Exposure Termination	1005 ± 60	926 ± 141	794 ± 60[b]
2 Week Postexposure	1496 ± 114	1169 ± 57[b]	1223 ± 43[b]
9 Month Postexposure	1078 ± 155	765 ± 80	799 ± 84

[a] Mean ± SE, N = 10.
[b] Statistically different from control value at $p < 0.05$.

Discussion

Previous 90-day continuous inhalation studies conducted by the THRU have shown microscopic changes in the kidneys of male rats exposed to a variety of hydrocarbon fuels. These studies have typically utilized an exposure termination sacrifice, an interim sacrifice at 19 months postexposure, and a final sacrifice at study termination. Often times the progress and extent of hydrocarbon induced kidney tubule changes during postexposure periods was masked by the renal tissue changes inherent in aged rats. The JP-8 protocol was designed to increase the frequency of tissue examinations between exposure termination and study termination, thus giving additional information pertaining to the progression of the renal injury.

Although histopathologic examination of the tissues collected to date in the study is not yet complete, the results available at exposure termination strongly suggest that renal damage was induced in male rats by JP-8 exposure. Trends toward decreased body weight gain, increased BUN and creatinine levels, and increased kidney weight have been noted in male rats exposed via inhalation to other hydrocarbon fuels. These trends were also evident in the JP-8 exposed male rats. In addition, urinalysis results at exposure termination indicated decreased concentrating ability along with the presence of epithelial cells, presumably resulting from renal tubular damage. Urinalysis results at 2 weeks and 9 months postexposure showing a reduction of epithelial cells in the urine of exposed male rats with normal osmolality suggest recovery upon removal from exposure.

TABLE 20. RAT ORGAN WEIGHTS 9 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO JP-8[a]

	Male		
	Control (N = 10)	500 mg/m^3 (N = 10)	1000 mg/m^3 (N = 10)
Fasted Body wt (g)	416 ± 10	405 ± 5	397 ± 6
Liver wt (g)	10.85 ± 0.31	10.54 ± 0.26	10.25 ± 0.30
Liver/100 g body wt	2.61 ± 0.03	2.60 ± 0.04	2.59 ± 0.07
Kidney wt (g)	2.65 ± 0.05	2.64 ± 0.05	2.79 ± 0.09
Kidney/100 g body wt	0.64 ± 0.01	0.65 ± 0.01	0.70 ± 0.02[b]
Spleen wt (g)	0.71 ± 0.02	0.71 ± 0.02	0.69 ± 0.01
Spleen/100 g body wt	0.17 ± 0.003	0.17 ± 0.003	0.18 ± 0.003

	Female		
	Control (N = 10)	500 mg/m^3 (N = 7)	1000 mg/m^3 (N = 10)
Fasted Body wt (g)	212 ± 3	218 ± 9	203 ± 4
Liver wt (g)	5.40 ± 0.16	5.38 ± 0.17	5.26 ± 0.15
Liver/100 g body wt	2.55 ± 0.06	2.49 ± 0.10	2.59 ± 0.05
Kidney wt (g)	1.51 ± 0.05	1.61 ± 0.06	1.50 ± 0.05
Kidney/100 g body wt	0.71 ± 0.02	0.75 ± 0.04	0.74 ± 0.02
Spleen wt (g)	0.45 ± 0.01	0.46 ± 0.02	0.44 ± 0.01
Spleen/100 g body wt	0.21 ± 0.003	0.22 ± 0.02	0.22 ± 0.01

[a] Values expressed as mean ± SE.
[b] Statistically different from control value at $p < 0.05$.

Consistent with previous hydrocarbon fuel inhalation studies, the male rats exposed to 1000 mg/m^3 JP-8 exhibit mildly reduced RBC counts with reduced hemoglobin and hematocrit levels, long after removal from the exposure.

There was no indication that JP-8 had any direct adverse effect on longevity in either species of rodents. However, JP-8

exacerbated the chronic dermatitis condition common in C57BL/6 mice resulting in more frequent moribund sacrifices among the exposed mice groups.

This study will be terminated in December 1984 at which time the remaining animals will be killed. Data collected at that time will be presented in the next annual report.

NINETY-DAY CONTINUOUS INHALATION EXPOSURE TO PETROLEUM JP-4 JET FUEL

Petroleum derived JP-4 was selected as part of a comparative series of studies on the chronic effects of inhaled hydrocarbon fuels. The exposure portion of this study began in August 1979 and continued for 90 days after which all dogs and one third of the rodents were necropsied to assess chronic toxicity effects in primary tissues. The remaining rodents were held for an additional 19 months at which time half the remaining rodents were killed. The final sacrifice of rodents took place at 21 months postexposure (two years on study). Each exposure and control group of animals consisted of 75 male and 75 female Fischer 344 rats, 150 female C57BL/6 mice and 6 purebred beagle dogs, equally divided by sex. The numbers of rodents used were selected to provide a statistically valid number of each species to reach the required age for tumor induction allowing for natural and toxicologic attribution.

Information on the experimental protocol, methodology of inhalation exposure, and clinical data obtained during the 90-day exposure phase was given in a previous annual report (MacEwen and Vernot, 1980). This report updates clinical observations during the postexposure observation period and presents histopathologic evaluation of the exposed animals necropsied for this purpose at the end of the 90-day exposure phase.

JP-4 is a complex mixture of aliphatic and aromatic hydrocarbon compounds defined in terms of physical and chemical characteristics and including various additives, all of which meet the requirements of Military Specification MIL-J-5624E.

The animal exposure concentrations selected, 1000 and 500 mg/m^3, were chosen after analysis of the benzene concentration of the JP-4 lot available. These concentrations of JP-4 insured

that the level of benzene in the chambers would not exceed that equivalent to a 6-hour time-weighted average (TWA) of 10 ppm.

Results

At the conclusion of the 90-day exposure period, indications of toxicity included depression in mean body weight in both sexes of rats, a dose related increase in kidney weights and creatinine levels in male rats. Clinical chemistry analysis of the dog blood showed a significant increase in globulin with a resultant increase in total protein values. Albumin values of all groups remained normal throughout the 13-week exposure period. The blood urea nitrogen values of the test dogs showed an increase over the values of the control dogs starting at two weeks and continuing through the termination of exposure. Although all three blood parameters were significantly different from the control values, all were within normal biological limits for the species.

The hematologic and clinical chemistry values of the male and female rats sacrificed after 19 months postexposure are shown in Tables 21 and 22. Scattered differences were noted in various blood parameters of both sexes of rats. The differences are sporadic and within normal limits for this species.

TABLE 21. MEAN HEMATOLOGIC AND CLINICAL CHEMISTRY VALUES OF MALE RATS 19 MONTHS AFTER EXPOSURE TO JP-4

	Controls (N=14-16)	500 mg/m³ (N=12)	100 mg/m³ (N=19)
RBC (10^6 cells/mm³)	7.1	7.5	7.0
WBC (10^3 cells/mm³)	5.6	5.5	11.1
HCT (%)	40.2	50.8	44.6
HGB (g/dl)	16.1	16.8	15.0
Total Prot. (g/dl)	7.7	7.6	7.4[a]
Albumin (g/dl)	3.7	3.6	3.3[b]
Globulin (g/dl)	4.1	4.0	4.0
A/G Ratio	0.91	0.92[a]	0.83[a]
Glucose (mg/dl)	141.2	156.0	142.3
Calcium (mg/dl)	11.5	11.4	11.2
Bilirubin (mg/dl)	0.46	0.51	1.55
Creatinine (mg/dl)	0.58	0.61	0.61
SGPT (IU/L)	43.1	39.2	36.5
SGOT (IU/L)	104.0	89.6	102.8
Alk. Phos. (IU/L)	10.1	7.3[b]	7.6[a]
BUN (mg/dl)	17.7	18.0	20.6
MCV	70.6	68.0	65.3
MCH	23.2	22.6	22.2
MCHC	32.8	33.3	33.9[a]

[a] Significantly different from controls, $p < 0.05$.
[b] Significantly different from controls, $p < 0.01$.

TABLE 22. MEAN HEMATOLOGIC AND CLINICAL CHEMISTRY VALUES OF FEMALE RATS 19 MONTHS AFTER EXPOSURE TO JP-4

	Controls (N=14, 17)	500 mg/m^3 (N=16, 18)	1000 mg/m^3 (N=16, 17)
RBC (10^6 cells/mm^3)	7.4	6.9	7.4
WBC (10^3 cells/mm^3)	4.2	8.6	3.8
HCT (%)	44.7	38.9[b]	43.4
HGB (g/dl)	14.7	13.5	15.0
Total Prot. (g/dl)	7.9	8.1	8.2[a]
Albumin (g/dl)	4.0	4.0	4.1
Globulin (g/dl)	4.0	4.1	4.1
A/G Ratio	1.0	1.0	1.0
Glucose (mg/dl)	157.0	145.0	148.6
Calcium (mg/dl)	11.2	11.2	11.3
Bilirubin (mg/dl)	0.49	0.76	0.53
Creatinine (mg/dl)	0.42	0.47[a]	0.42
SGPT (IU/L)	49.1	51.4	44.6
SGOT (IU/L)	92.0	136.6	78.3
Alk. Phos. (IU/L)	7.2	8.0	6.2
BUN (mg/dl)	13.6	14.4	14.0
MCV	61.5	58.9	58.8
MCH	20.1	20.4	20.3
MCHC	32.9	34.7[b]	34.5[b]

[a] Significantly different from controls, $p < 0.05$.
[b] Significantly different from controls, $p < 0.01$.

The body weight curves of male and female rats are shown in Figures 6 and 7. Both groups of JP-4 exposed male rats had depressed weight gains compared to unexposed male rats during the first six months of the study. This difference again became evident at 12 months and continued through study termination. Mean weights of the control female rats were significantly less than the two exposed groups through the first 17 months of the study. At this point the control group surpassed the test groups, remaining higher until the completion of the study.

Organ weights for the male and female rats sacrificed after 19 months postexposure are shown in Tables 23 and 24, respectively. Because the body weights of the male rats were not measured, it was not possible to determine ratios. However, no statistically significant differences were found in mean organ weights of the test male rats when compared to the controls.

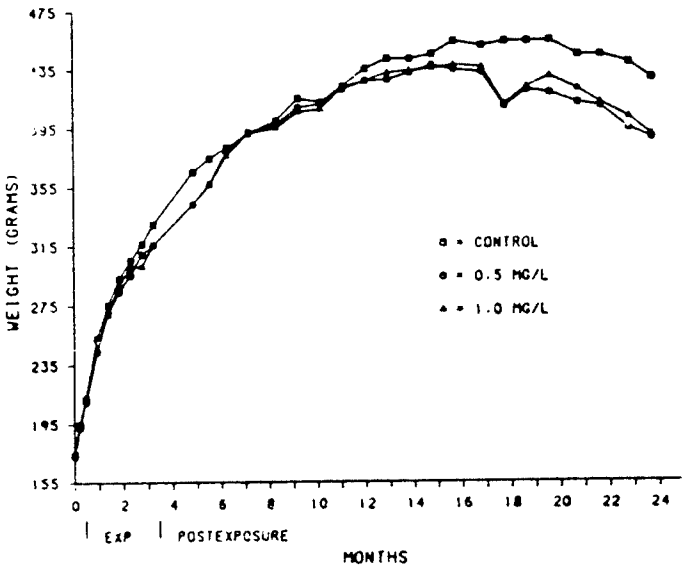

Figure 6. Effect of 90-day continuous inhalation exposure to Petroleum JP-4 vapor on male rat body weight.

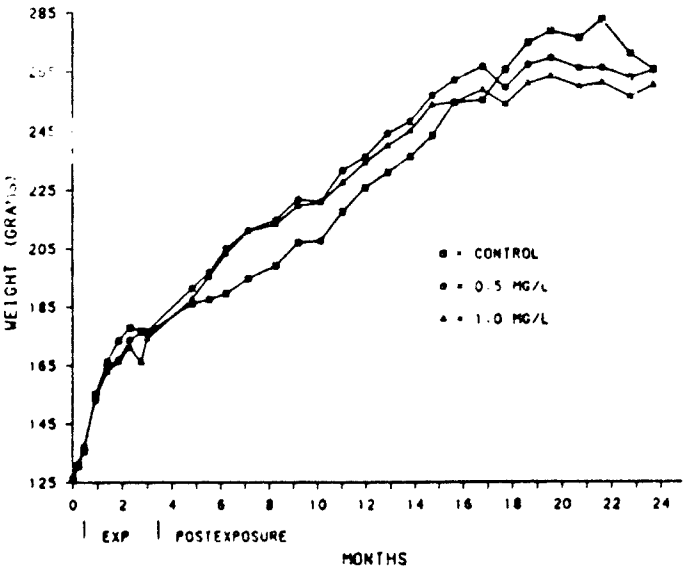

Figure 7. Effect of 90-day continuous inhalation exposure to Petroleum JP-4 vapor on female rat body weight.

TABLE 23. MALE RAT ORGAN WEIGHTS[a] 19 MONTHS AFTER 90-DAY EXPOSURE TO JP-4 VAPOR

	Controls	500 mg/m^3	1000 mg/m^3
Liver wt, g	11.50 ± 1.70	10.95 ± 1.36	11.93 ± 1.79
Spleen wt, g	1.11 ± 0.50	0.99 ± 0.24	1.93 ± 1.94
Kidney wt, g	2.73 ± 0.34	2.66 ± 0.23	2.94 ± 0.35

[a] Mean ± SE, N = 18-20.

TABLE 24. FEMALE RAT ORGAN WEIGHTS[a] 19 MONTHS AFTER 90-DAY EXPOSURE TO JP-4 VAPOR

	Controls	500 mg/m^3	1000 mg/m^3
Body wt, g	279.0 ± 31.9	250.2[b] ± 4.7	252.6[b] ± 19.9
Liver wt, g	6.95 ± 1.11	7.04 ± 1.07	6.69 ± 0.73
Liver/100 g body wt	2.49 ± 0.24	2.83[a] ± 0.47	2.65 ± 0.25
Spleen wt, g	0.55 ± 0.26	1.68 ± 2.98	0.65 ± 0.70
Spleen/100 g body wt	0.19 ± 0.07	0.69 ± 1.22	0.26 ± 0.29
Kidney wt, g	1.90 ± 0.20	1.92 ± 0.14	1.87 ± 0.16
Kidney/100 g body wt	0.68 ± 0.07	0.77[b] ± 0.08	0.74[c] ± 0.05

[a] Mean ± SE, N = 17-20.
[b] Different from controls at 0.01 level of confidence.
[c] Different from controls at 0.05 level of confidence.

Statistically significant differences were found in the weights of livers and kidneys as a percentage of body weight in the female rat group, but the differences are not dose related. The high mean spleen weight of the female group exposed to 500 mg/m^3 reflects enlarged spleens in two of the 16 rats examined. The mean weight of the spleens from the remaining 14 rats was within normal limits.

Incidence rates of micropathologic tissue lesions seen in the animals sacrificed at the end of the 90-day continuous exposure to JP-4 vapors are listed in Tables 25-27 for dogs, rats, and

mice, respectively. The numbers of animals examined include those that died during the exposure period as well as those from the planned sampling groups.

TABLE 25. LESIONS SEEN IN MALE AND FEMALE BEAGLE DOGS FOLLOWING 90-DAY CONTINUOUS EXPOSURE TO JP-4 VAPOR

	Unexposed Controls		500 mg/m³ Exposed		1000 mg/m³ Exposed	
	M	F	M	F	M	F
Lung						
Nematodiasis	0/3	0/3	0/3	2/3	0/3	0/3
Eosinophilic granuloma	1/3	0/3	1/3	2/3	0/3	1/3
Alveolar inflammation	2/3	1/3	1/3	2/3	0/3	1/3
Alveolar nematodiasis	0/3	0/3	0/3	0/3	0/3	1/3
Nasal mucosa						
Inflammation	2/3	3/3	2/3	2/3	3/3	1/3
Lymph node						
Mesenteric, hemorrhage	0/3	3/3	3/3	3/3	0/3	0/3
Heart						
Endocardiosis	0/3	1/3	0/3	2/3	2/3	0/3
Kidney						
Mineralization renal papilla	2/3	3/3	1/3	2/3	3/3	2/3
Collecting tubule cast	2/3	2/3	1/3	2/3	1/3	1/3
Convoluted tubules pigmentation	2/3	0/3	0/3	0/3	1/3	0/3

All microscopic lesions observed in canine subjects are regarded as spontaneous changes or natural diseases found in laboratory beagles. The most significant findings were mild to moderate inflammatory changes in the lungs and associated lymphoid tissues. These changes were not dose dependent, and in most cases were compatible with infestations by canine lungworms, Filaroidea sp.

The most significant lesions seen were confined to the kidney of male rats. In this group, hyaline degeneration of the renal tubular epithelium was present in 100% of the high dose group and 96% of the low dose group, as opposed to only 8% of the controls. This lesion consisted of the formation of hyaline

crystalloid intracytoplasmic inclusions. The exact nature of this material is not known but probably represents excessive cytoplasmic accumulation of proteins reabsorbed from the glomerular filtrate. Also, renal tubular casts were present to the same degree in both groups of test rats but were not observed in the control rats. The casts were composed of necrotic exfoliated tubular epithelial cells which formed prominent tubular plugs near the corticomedullary junction and resulted in focal tubular dilatation.

TABLE 26. LESIONS SEEN IN MALE AND FEMALE FISCHER 344 RATS FOLLOWING 90-DAY CONTINUOUS EXPOSURE TO JP-4 VAPOR

	Unexposed Controls		500 mg/m^3 Exposed		1000 mg/m^3 Exposed	
	M	F	M	F	M	F
Nasal mucosa						
Inflammation	5/26	3/25	0/25	0/25	0/25	0/25
Lungs and bronchi						
Lymphocytic inflammatory infiltrate	17/26	4/24	13/27	8/25	12/27	9/25
Kidneys						
Renal tubules casts	0/26	0/25	26/27[a]	0/25	27/27[a]	1/25
Tubule degeneration, hyaline	2/26	0/25	26/27[a]	0/25	27/27[a]	1/25

[a] Significant at the 0.01 level.

In female rats, lymphocytic inflammatory infiltrates were more prevalent in exposed animals. However, this is a common change seen in most groups of aging rats. The presence of this change in female rats is not considered significant.

Hepatocellular fatty change is the most significant finding in female mice. This lesion was found in a high percentage of exposed mice as opposed to control mice and was most prominent in the centrilobular region of the liver. Microscopically, the lesion consisted of multiple, discrete vacuoles of varying sizes within the cytoplasm of hepatocytes. This change is considered to be a degenerative process and is regarded as reversible if there is no further damage to the cell.

TABLE 27. LESIONS SEEN IN FEMALE MICE FOLLOWING 90-DAY CONTINUOUS EXPOSURE TO JP-4 VAPOR

	Unexposed Controls	500 mg/m^3 Exposed	1000 mg/m^3 Exposed
Lungs and bronchi			
Lymphocytic inflammatory infiltrate	10/50	6/47	10/45
Inflammation	4/50	2/47	1/45
Liver			
Focal cellular change	1/49	9/48[a]	3/45
Sinisoidal hematopoiesis	3/49	7/48	11/45[b]
Hepatocyte fatty change	3/49	42/48[a]	40/45[a]
Gallbladder and bile ducts			
Acute inflammation	4/45	14/42[a]	8/39
Hyaline degeneration	2/45	4/42	6/39
Kidneys			
Tubule dilatation	4/45	13/48[b]	4/45

[a] Significant at the 0.01 level.
[b] Significant at the 0.05 level.

Acute inflammatory changes consisting of infiltrates of eosinophils in the submucosa of the gallbladder were seen with slightly greater frequency in exposed female mice when compared with controls. Frequently, but not exclusively, this change was accompanied by hyaline degenerative changes of the mucosal epithelium. These findings are common in older C57BL/6 mice and because the increased incidence in exposed subjects was not dose dependent, this change was not thought to be exposure related.

Renal tubular dilatation was diagnosed with slightly greater frequency in the low dose mice (27%) when compared with the controls (9%) and high dose (8%) animals. This lesion consisted of slightly dilated tubules near the corticomedullary junction which were filled with pink, homogeneous fluid. This fluid was thought to originate from an incompetent glomerular filtration mechanism secondary to mild membraneous glomerulonephritis. Glomerulonephritis is common in aging mice.

Kidney tissue was collected from two male rats in each exposure group for electron microscopic (EM) evaluation. Two of the kidneys from each group were fixed by vascular perfusion while the remaining kidneys were fixed by immersion.

Hyaline, crystalloid intracytoplasmic inclusions were present in proximal tubule cells. Inclusions appeared to be larger and more numerous in the second segment (S2) (Figure 8) of the proximal tubules (PT) than in the first segment (S1) (Figure 9) in both low dose and high dose groups. The severity of the inclusions was greater in the high dose group although several low dose PT cells contained crystalloid inclusions equal in size and number to high dose PT (Figure 10).

Intratubular casts observed near the corticomedullary junction by light microscopy were seen by EM to be composed of necrotic, exfoliated tubular epithelial cells (Figure 11). The prominent components that were distinguishable in necrotic casts included degenerating mitochondria and assorted cellular membranes, especially vesiculated endoplasmic reticulum (ER). These casts compressed and distorted viable tubular lining cells to varying degrees. In one photomicrograph (Figure 12) where debris was thought to represent early cast formation in the S2 segment, there was minimal to mild compression of lining cells and microvilli. In other photographs (e.g., Figure 11) casts exhibited greater density and marked compression and attenuation of lining cells. These more mature casts were believed to be at the juncture of the outer and inner stripe of the outer medulla where the pars recta of the proximal convoluted tubule becomes the descending limb of Henle's loop. In order to positively identify the segment of PT in which formation of tubular casts occurs, further studies are necessary.

Mitochondria and ER were not affected until PT cells: 1) became engorged with inclusions; 2) exhibited excessive dilatation of the abluminal cell membrane; or 3) began to form a cast. The appearance of the mitochondria then became more irregular in shape and the cristae appeared more dilated than in the control group (Figures 10, 11, and 12). This mild swelling of the mitochondria was occasionally present in PT and distal tubules that otherwise appeared normal. Initially, ER was normal to mildly dilated and became vesiculated in later stages of the more extensively affected PT cells. ER was normal in distal tubules. Distal tubules and glomeruli appeared normal in both low and high dose groups (Figures 13 - 15).

Figure 8. S2 segment proximal tubule cell filled with crystalloid intracytoplasmic inclusions (12,000 x).

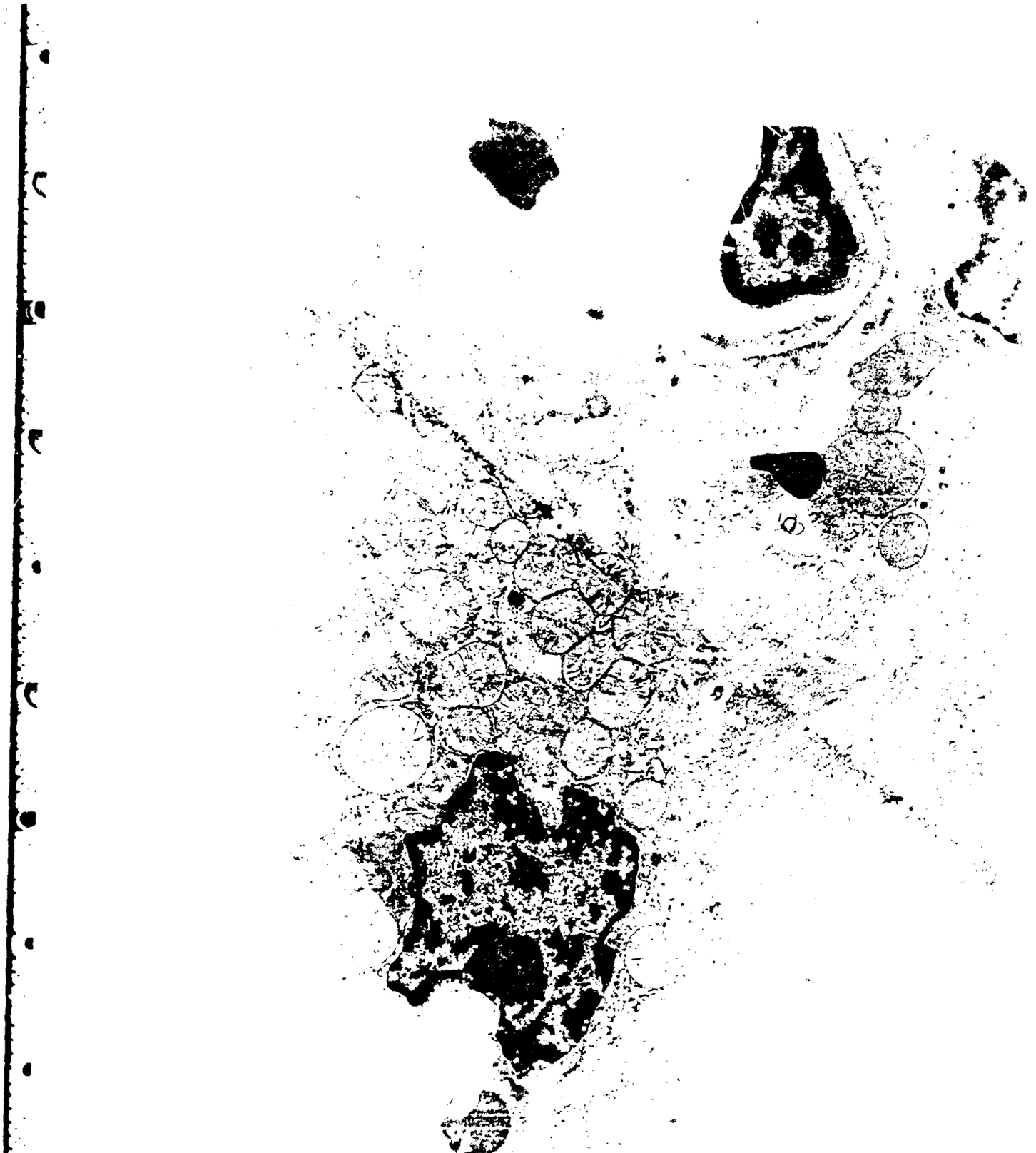

Figure 9. S1 segment proximal tubule cell is continuous with glomerulus in lower left of photomicrograph (12,000 x).

Figure 10. Proximal tubule cell from low dose group filled with crystalloid intracytoplasmic inclusions (17,000 x).

Figure 11. Tubular cast filled with necrotic debris (15,000 x).

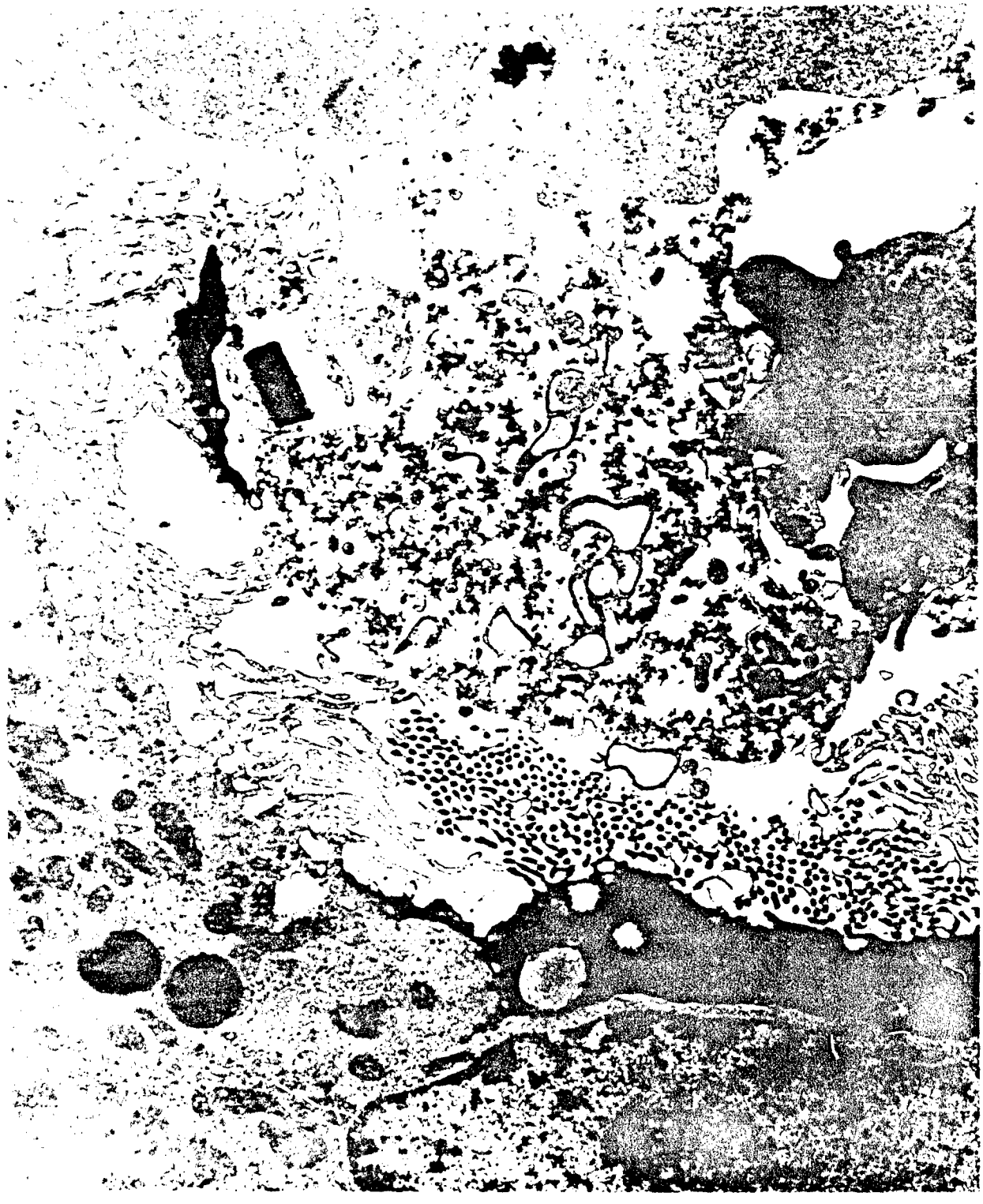

Figure 12. Possible S2 segment of proximal tubule filled with necrotic debris that may be in an early stage of cast formation (17,000 x).

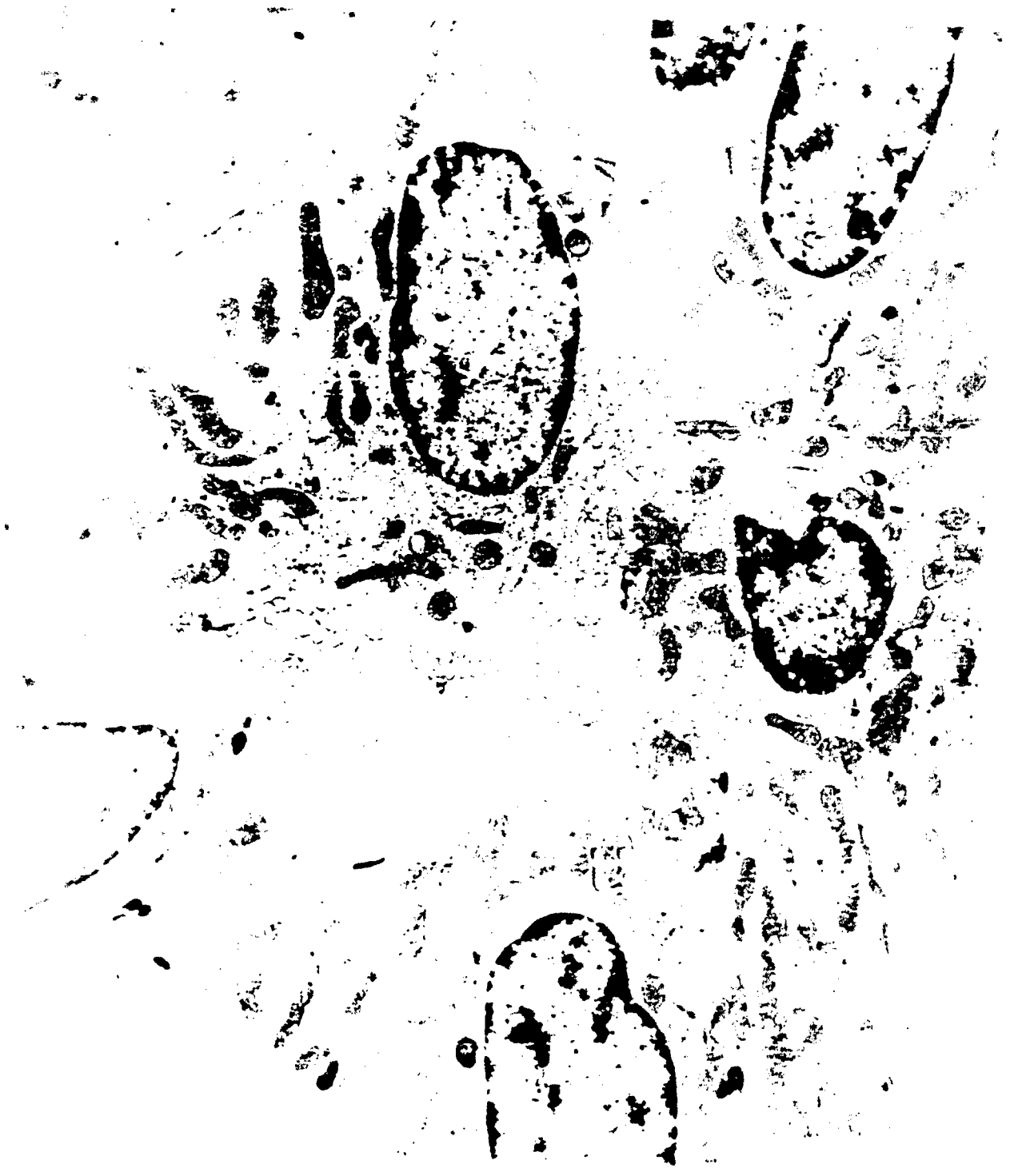

Figure 13. Normal distal tubule composed of normal cells (10,000 x).

Figure 14. Portion of nonperfused glomerulus from low dose group showing several capillaries with a red blood cell in the largest (12,000 x).

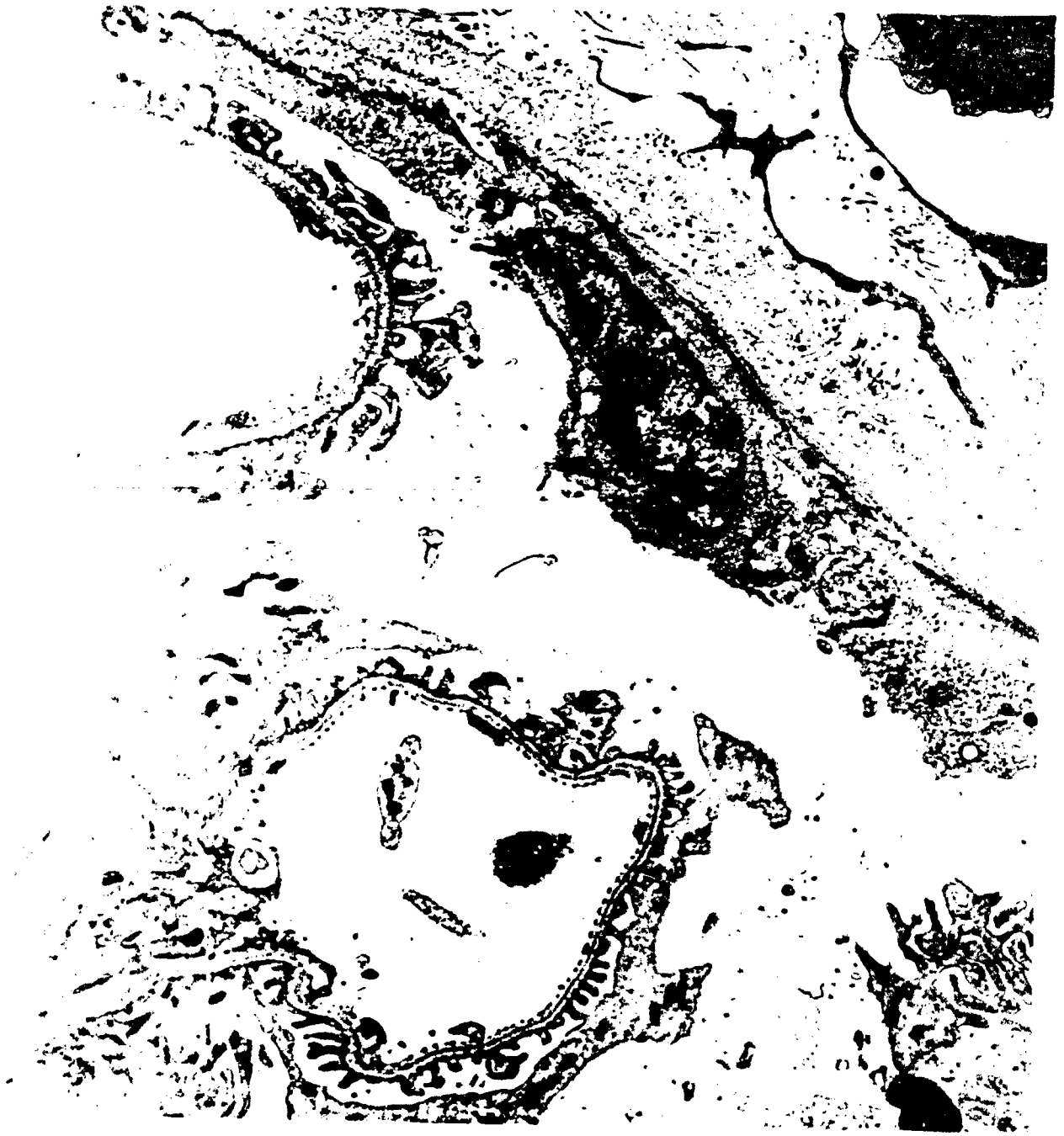

Figure 15. Portion of perfused glomerulus from high dose group showing two capillaries and prominent Bowman's space (12,000 x).

The examination of tissue from the animals that died during the postexposure observation period or were killed at study termination is not yet complete and will be discussed in a future report.

NINETY-DAY CONTINUOUS INHALATION EXPOSURE OF RATS AND MICE TO SHALE JP-4 JET FUEL

The Air Force has embarked on a test and evaluation program to ensure the compatibility of shale derived turbine fuels with aircraft systems when introduced operationally at Air Force bases in late 1983. Shale derived JP-4 is included in this program.

Since the first test fuel supplied by Geokinetics Corporation and the fuel to be used later in the operational validation program will be produced from different shale crudes and at two different refineries, acute and subchronic toxicity studies will be accomplished with both fuels. Studies performed with the Geokinetics JP-4 test fuel will provide the data base for environmental and health assessments at the first operational base and will allow comparison of source variability on bioassay results. Longer term oncogenic studies will be conducted on the operational validation fuel to assess the chronic toxicity and the carcinogenic properties of a fuel derived and processed differently from existing aircraft hydrocarbon fuels.

Previous studies of hydrocarbon fuels have shown histopathologic changes in the kidneys of male rats at exposure termination (MacEwen and Vernot, 1978; 1981). Due to the similarity of the fuels, it is highly likely that renal damage will result in male rats exposed to Shale JP-4. In order to further evaluate this renal toxicity, routine urinalysis and serial sacrifices of male rats for tissue examination have been included in this study. Sampling in this manner will allow for microscopic evaluation of kidney tissue alterations prior to onset of chronic nephropathy common to older rats.

This study was designed to determine the toxic effects of a 90-day continuous exposure of rats and mice to Shale derived JP-4 vapors for comparison with the previous 90-day Petroleum JP-4 study (MacEwen and Vernot, 1980). The conditions were selected to conform with the other fuel studies conducted in the THRU laboratory.

The JP-4 Shale fuel used was a complex mixture of aliphatic and aromatic hydrocarbon compounds including various additives. Minor revisions from Petroleum JP-4 military specifications were made to the procurement specification requirements. The are:

Aromatic content (min) - 9% (by volume)
Nitrogen (max) - 20 ppm by weight

Mice and rats were exposed to 500 mg/m^3 and 1000 mg/m^3 Shale JP-4 vapor on a continuous basis for 90 days. For these purposes, two chambers were utilized. Sham exposed controls were maintained in a separate but identical chamber. Each chamber housed 95 male and 75 female Fischer 344 rats and 100 male and 100 female C57BL/6 mice.

Following the 90-day continuous exposure period, 15 rats of each sex and 25 mice of each sex from each group were killed for evaluation of exposure effects. Additional interim sacrifices were scheduled for 2 weeks postexposure (10 male rats), 2 months postexposure (10 male rats), and 9 months postexposure (10 male and 10 female rats; 10 male and 10 female mice).

The remaining animals will be killed during the 24th month of the study. Should the mortality of any one group reach 90% prior to the 24th month of the study, all of the remaining animals of that sex and species from all groups will be sacrificed. The selection of animals to be killed at each sampling period was determined by a randomization list prepared by the UCI/THRU Statistics Department.

The contaminant introduction system for Shale JP-4 was similar to the systems used for the previous fuel studies. The liquid material was pumped under low pressure from a 55-gallon supply drum. It passed through flowmeters to glass evaporator columns heated to an air temperature not greater than 135°F. The air stream flowing through the evaporator carried the vapors into the main air supply for the domes. Excess fuel which was not vaporized in the evaporator was drained into the receiving tank and collected for disposal.

Thermocouples were placed at the top and bottom of the glass evaporator to sense any hazardous increase in temperature and to activate both an alarm and a solenoid valve system which would cut off the fuel supply.

Detailed figures of the vapor introduction system, the alarm and emergency shut down system, and the analysis system have been reported in a previous annual report (MacEwen and Vernot, 1980).

Analysis of the chamber concentrations was done by pumping air samples from each exposure chamber into a total hydrocarbon analyzer. During previous testing of fuels by this laboratory, we found that hexane had a similar hydrocarbon detector sensitivity as the fuels. Therefore, known hexane concentrations were used as calibration standards. A weekly span check of the hydrocarbon analyzer was made using a prepared standard bag of hexane at an appropriate concentration. Full calibration at a range of concentrations bracketing the exposure concentrations was conducted monthly or whenever the weekly span check indicated a change in the analytical system.

Output of the vapors by the generation system was a function of the fuel itself, fuel flow rate, air flow rate, and temperature. Under defined operating conditions, the output was stable; therefore, hourly checks were made to assure that the predetermined settings were maintained.

Gas chromatographic fingerprints were obtained from a liquid sample of each drum of Shale JP-4 prior to the initiation of the study to insure that sufficient uniform material was available for completion of the 90-day exposure. A GC fingerprint of a sample of each drum was also obtained as the drum was introduced into the exposure to insure constancy of the Shale JP-4 over the course of the exposure. GC fingerprints of the contaminant in the chamber were also obtained every two weeks. A Royco® particle counter equipped with a digital monitor was used to measure possible formation of vapor condensate aerosol.

All animals were observed hourly during the exposure and are now being observed daily until the mortality rate warrants more frequent examinations. At that time, cage group size will be reduced and observations will be increased to 6/day at 4-hour intervals.

Rats were individually weighed at biweekly intervals during exposure and are being weighed monthly during the postexposure period. Mice were weighed in groups with the group mean weights being followed on a monthly basis throughout the experimental period.

The tests listed in Table 28 were performed on all rats sacrificed at exposure phase termination. The tests will also be made on the rats sacrificed at 9 months postexposure and on 10 male and 10 female rats from each group at the end of the study. Blood samples for hematology, creatinine, and BUN measurements were taken from the 10 male rats killed at 2 weeks and 2 months postexposure.

TABLE 28. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON RATS EXPOSED TO SHALE JP-4 JET FUEL VAPOR

Hematology	Chemistry
Hematocrit	Sodium
Hemoglobin	Potassium
RBC	Calcium
WBC	Albumin/Globulin
Differentials	Total Protein
Mean Corpuscular Volume (MCV)	Glucose
Mean Corpuscular Hemoglobin (MCH)	Alkaline Phosphatase
Mean Corpuscular Hemoglobin Concentration (MCHC)	SGPT
	SGOT
	Bilirubin
	Creatinine
	BUN

Urine samples were collected preexposure from 10 male rats in each group for the tests shown in Table 29. Urine samples will be collected from similar numbers of male rats at each scheduled sacrifice.

TABLE 29. URINALYSIS TESTS PERFORMED ON MALE RATS EXPOSED TO SHALE JP-4 JET FUEL VAPOR

pH	Blood
Protein	Nitrate
Glucose	Urobilinogen
Ketone	Osmolality
Bilirubin	Microscopic

At the completion of the 90-day exposure period, 10 randomly selected male rats each from the control group and the 1000 mg/m^3 Shale JP-4 exposed group were placed in metabolism cages for

urine collection. This urine was used for GC/MS analysis of metabolites. The urine was extracted into ethyl ether before and after hydrolysis of glucuronides and sulfates and then injected into the GC/MS.

All animals that died or were sacrificed during exposure or on completion of exposure were necropsied and tissues collected as shown in Table 30. Histopathologic examination will occur on the tissues listed in Column 1 of Table 30 obtained from these animals. The tissues taken which appear in Column 2 of Table 30 will be stored and examined only if warranted by other observations. Histopathologic examination will occur on the tissues listed in both columns of Table 30 obtained from all of the rodents that die or are sacrificed during the postexposure phase of the study. Two of the rodents of each sex from each group had organs perfused and tissue collected for electron microscopic (EM) examination at the end of the exposure phase.

TABLE 30. TISSUES SAMPLED FROM ANIMALS EXPOSED TO SHALE JP-4 JET FUEL VAPOR

Column 1	Column 2
Gross lesions	Skin
Tissue masses or suspect tumors and regional lymph nodes	Mandibular lymph node
Larynx	Mammary gland
Trachea	Salivary gland
Lungs and bronchi	Stomach
Heart	Duodenum
Thyroid	Ileum
Parathyroid	Colon
Esophagus	Anus
Liver	Mesenteric lymph node
Sternebrae, vertebrae or femur (plus marrow)	Thigh muscle
Spleen	Sciatic nerve
Kidneys	Seminal vesicles
Bladder	Prostate
Nasal cavity	Testes
Brain	Ovaries
Mandibular lymph node (mice only)	Uterus
Mesenteric lymph node (mice only)	
Thymus	
Gallbladder	
Pancreas	
Adrenals	
Pituitary	

Whole body, liver, kidney, and spleen weights were or will be measured from all rats killed at the sacrifices scheduled at exposure termination, 9 months postexposure, and study termination.

Data from routine animal weighing, hematology, urinalysis, blood chemistry, and organ weighing are analyzed for statistical significance using the Student's t-test. Histopathology lesions are analyzed using the Fisher Exact test.

Analysis of the vapor-air mixtures resulted in mean measured concentrations within one-tenth percent of the planned nominal values as shown in Table 31. No outward signs of toxic stress were observed in either species and no exposure related deaths occurred during the 90-day study. Two rats in the low concentration chamber escaped from their exposure cage during the study and were accidently killed.

TABLE 31. ANALYSIS OF SHALE JP-4 VAPOR CONCENTRATIONS INHALED BY MALE AND FEMALE RATS AND MICE FOR 90 DAYS

Nominal Concentration, mg/m^3	500.0	1000.0
Mean Concentration, mg/m^3	499.6	1001.0
Standard Deviation	6.0	10.9
Standard Error	0.6	1.1
Lowest Daily Average, mg/m^3	461.0	929.0
Highest Daily Average, mg/m^3	506.0	1021.0

Exposure to Shale JP-4 vapors affected the body weight gains of both the male and female rats (Figures 16 and 17). The differences were noticeable throughout the exposure period in both sexes.

A summary of the organ weights of male and female rats is given in Table 32. Significant dose related differences in mean liver and kidney weights were noted in both groups of Shale JP-4 exposed male rats. The same two parameters were significant when considered as a percentage of body weight. Mean spleen weights were significantly higher in the 500 mg/m^3 JP-4 exposed group only.

The female organ weights were relatively unaffected. Only those exposed to the high level showed a slight but significant difference in the liver to body weight ratios.

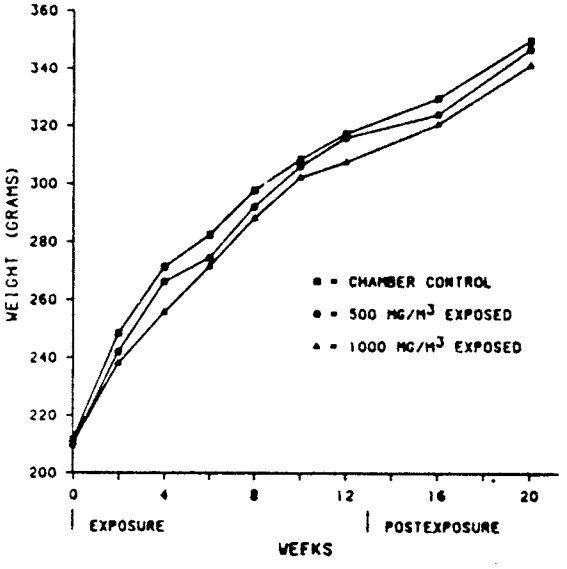

Figure 16. Effect of 90-day continuous inhalation exposure to Shale JP-4 vapor on male rat body weight.

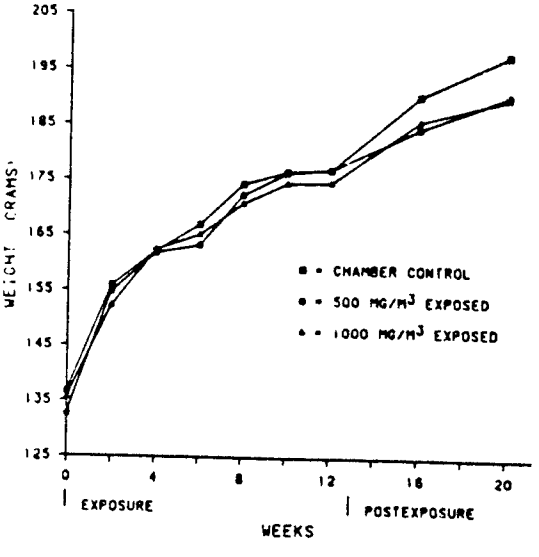

Figure 17. Effect of 90-day continuous inhalation exposure to Shale JP-4 vapor on female rat body weight.

TABLE 32. SUMMARY OF BODY AND ORGAN WEIGHTS OF FISCHER 344 RATS EXPOSED TO SHALE JP-4 VAPORS FOR 90 DAYS

Treatment Group	Mean Weight in Grams				% of Body Weight		
	Body	Spleen	Liver	Kidney	Spleen	Liver	Kidney
Male Rats							
Control	301.2	0.550	8.278[a]	2.168	0.183	2.768	0.724
500 mg/m^3	306.5	0.613[a]	9.352	2.689[a]	0.200[a]	3.047[a]	0.876[a]
1000 mg/m^3	295.7	0.574	9.478[a]	2.874[a]	0.194	3.207[a]	0.972[a]
Female Rats							
Control	173.5	0.417	4.817	1.372	0.240	2.757	0.785
500 mg/m^3	171.5	0.412	5.005	1.377	0.240	2.934	0.810
1000 mg/m^3	164.3[a]	0.389	4.914	1.379	0.237	2.981[b]	0.837

[a] Different from control value, $0.001 < p < 0.01$.
[b] Different from control value, $0.01 < p < 0.05$.

Mean blood values from both male and female rats, sacrificed at the termination of exposure, are summarized in Tables 33 and 34, respectively. No biologically significant difference in any parameter was noted among the groups.

The results of the analyses of blood taken from male rats two weeks postexposure are shown in Table 35. A statistically significant difference exists in the BUN values of both groups of exposed rats when compared to the control group of rats. Again, these values are well within normal ranges and the significant difference is probably a reflection of the low-normal control mean values.

A significant dose related decrease in urine osmolality was seen in both Shale JP-4 exposed male rat groups when compared to unexposed controls at exposure termination (Table 36). This effect was still evident in urine samples taken at two weeks and two months postexposure from male rats.

TABLE 33. MALE FISCHER 344 RAT BLOOD PARAMETERS[a] AFTER CONTINUOUS EXPOSURE TO SHALE DERIVED JP-4 VAPORS FOR 90 DAYS

	Control	500 mg/m³	1000 mg/m³
WBC ($x10^3$ cells/mm^3)	5.8 ± 0.3	6.7 ± 0.3	6.2 ± 0.3
RBC ($x10^6$ cells/mm^3)	8.48 ± 0.15	8.26 ± 0.11	8.29 ± 0.11
HGB (g/dl)	15.3 ± 0.2	14.7 ± 0.2	14.7 ± 0.2
HCT (%)	41.6 ± 0.7	40.6 ± 0.6	41.1 ± 0.6
MCV (μm^3)	49.1 ± 0.1	49.1 ± 0.2	49.6 ± 0.1
MCH (pg)	16.0 ± 0.1	17.8 ± 0.1	17.7 ± 0.1
MCHC (g/dl)	36.7 ± 0.2	36.3 ± 0.2	35.7 ± 0.2[b]
Glucose (mg/dl)	214 ± 17	194 ± 13	185 ± 2
Tot. Prot. (g/dl)	6.9 ± 0.12	7.0 ± 0.08	7.11 ± 0.07
Albumin (g/dl)	0.83 ± 0.01	0.83 ± 0.02	0.89 ± 0.01
Globulin (g/dl)	6.14 ± 0.11	6.21 ± 0.08	6.23 ± 0.06
A/G Ratio	0.14 ± 0.0	0.13 ± 0.0	0.14 ± 0.0
BUN (mg/dl)	15.6 ± 0.9	16.9 ± 0.9	17.7 ± 0.5
Creatinine (mg/dl)	0.6 ± 0.03	0.7 ± 0.02	0.7 ± 0.02
Calcium (mg/dl)	10.7 ± 0.2	11.0 ± 0.2	11.0 ± 0.2
SGOT (IU/L)	102 ± 4	97 ± 3	88 ± 3
SGPT (IU/L)	52 ± 5	45 ± 2	43 ± 5
Alk. Phos. (IU/L)	96 ± 3	103 ± 3	95 ± 3
Bilirubin (mg/dl)	0.2 ± 0.03	0.2 ± 0.03	0.2 ± 0.04

[a] Values expressed as mean ± SE.
[b] Different from control value, $0.001 < p < 0.01$.

TABLE 34. FEMALE FISCHER 344 RAT BLOOD PARAMETERS[a] AFTER CONTINUOUS EXPOSURE TO SHALE DERIVED JP-4 VAPORS FOR 90 DAYS

	Control	500 mg/m³	1000 mg/m³
WBC ($x10^3$ cells/mm^3)	6.1 ± 0.3	5.8 ± 0.4	5.1 ± 0.5
RBC ($x10^6$ cells/mm^3)	7.61 ± 0.22	8.16 ± 0.24	8.34 ± 0.17
HGB (g/dl)	15.1 ± 0.3	15.0 ± 0.7	15.1 ± 0.3
HCT (%)	39.7 ± 1.2	42.8 ± 1.3	44.4 ± 1.1
MCV (μm^3)	52.2 ± 0.1	52.4 ± 0.1	53.2 ± 0.3[b]
MCH (pg)	20.1 ± 0.8	18.3 ± 0.5	18.1 ± 0.1
MCHC (g/dl)	38.4 ± 1.5	34.9 ± 1.0	34.1 ± 0.3
Glucose (mg/dl)	140 ± 6	130 ± 6	124 ± 7
Tot. Prot. (g/dl)	7.32 ± 0.08	7.47 ± 0.10	7.44 ± 0.17
Albumin (g/dl)	0.87 ± 0.01	0.92 ± 0.02	0.93 ± 0.02
Globulin (g/dl)	6.43 ± 0.07	6.55 ± 0.09	6.52 ± 0.15
A/G Ratio	0.14 ± 0.0	0.13 ± 0.0	0.14 ± 0.0
BUN (mg/dl)	16.9 ± 0.5	16.1 ± 0.4	15.9 ± 0.6
Creatinine (mg/dl)	0.6 ± 0.02	0.6 ± 0.02	0.5 ± 0.02
Calcium (mg/dl)	10.8 ± 0.1	10.8 ± 0.2	10.5 ± 0.2
SGOT (IU/L)	99 ± 2	85 ± 3	90 ± 9
SGPT (IU/L)	45 ± 2	42 ± 2	40 ± 2
Alk. Phos. (IU/L)	59 ± 2	58 ± 2	65 ± 6
Bilirubin (mg/dl)	0.3 ± 0.02	0.3 ± 0.01	0.4 ± 0.06

[a] Values expressed as mean ± SE.
[b] Different from control value, $0.001 < p < 0.01$.

TABLE 35. MALE FISCHER 344 RAT BLOOD PARAMETERS[a] TWO WEEKS FOLLOWING 90-DAY CONTINUOUS EXPOSURE TO SHALE DERIVED JP-4 VAPORS (N = 10)

	Control	500 mg/m^3	1000 mg/m^3
WBC (x10^3 cells/mm^3)	6.5 ± 0.3	7.7 ± 0.3	8.1 ± 0.4[b]
RBC (x10^6 cells/mm^3)	8.26 ± 0.25	8.06 ± 0.15	7.99 ± 0.23
HGB (g/dl)	15.2 ± 0.5	14.9 ± 0.3	15.1 ± 0.3
HCT (%)	41.2 ± 1.3	39.9 ± 1.0	40.0 ± 1.3
MCV (μm^3)	49.8 ± 0.2	49.4 ± 0.4	50.0 ± 0.3
MCH (pg)	18.3 ± 0.2	18.4 ± 0.1	18.9 ± 0.3
MCHC (g/dl)	36.8 ± 0.4	37.3 ± 0.2	37.9 ± 0.7
BUN (mg/dl)	14.7 ± 0.3	18.7 ± 0.6[c]	17.9 ± 0.8[c]
Creatinine (mg/dl)	0.7 ± 0.03	0.7 ± 0.03	0.7 ± 0.03

[a] Values expressed as mean ± SE.
[b] Different from control value, 0.001 < p < 0.01.
[c] Different from control value, 0.01 < p < 0.05.

TABLE 36. MEAN OSMOLALITY VALUES OF URINE FROM MALE FISCHER 344 RATS CONTINUOUSLY EXPOSED TO SHALE JP-4 VAPORS (N = 10)

	Osmolality[a]		
Treatment	0 days	90 days	2 wks post
Control	1497 ± 100	1197 ± 109	1944 ± 206
500 mg/m^3	1877 ± 174	882[b] ± 93	1129[c] ± 91
1000 mg/m^3	1645 ± 208	785[c] ± 69	1126[c] ± 59

[a] Mean ± SE (expressed as milliosmols/liter).
[b] Different from control value, 0.01 < p < 0.05.
[c] Different from control value, 0.001 < p < 0.01.

Table 37 shows the urine pH values of the male rats at each sampling period. The pH values of all groups changed in the same manner over the exposure and postexposure periods. Therefore, inhalation of Shale JP-4 vapors did not affect rat urine pH adversely.

This study is scheduled for termination in December 1985. Subsequent annual reports will contain additional experimental data.

TABLE 37. MEAN URINE pH VALUES FOR MALE FISCHER 344 RATS EXPOSED TO SHALE JP-4 VAPORS (N = 10)

Treatment	pH Value[a]			
	0 days	90 days	2 wks post	2 mos. post
Control	7.30 ± 0.15	6.75 ± 0.13	6.55 ± 0.05	8.30 ± 0.81
500 mg/m^3	7.25 ± 0.15	6.95 ± 0.16	6.70 ± 0.11	8.10 ± 0.15
1000 mg/m^3	7.60 ± 0.15	7.05 ± 0.16	6.85[b] ± 0.08	8.45 ± 0.50

[a] Mean ± SE.
[b] Different from control value, $0.01 < p < 0.05$.

EVALUATION OF STRAIN SUSCEPTIBILITY TO CHRONIC NEPHRITIS IN RATS EXPOSED TO SHALE DERIVED JP-4 JET FUEL

Previous subchronic inhalation studies with hydrocarbon vapors, including petroleum and shale derived jet fuels, have shown a pattern of toxic nephropathy in male rats (Carpenter et al., 1975a, 1975b, 1975c, 1975d; Gaworski et al., 1979a, 1979b, 1982, Phillips, 1982, and Bruner and Pitts, 1982). The lesions were described as greatly accentuated hyaline droplets in proximal tubular epithelium and dilated, cystic tubules near the corticomedullary junction which were plugged with necrotic cellular debris. This lesion has not been reported in female rats exposed to hydrocarbon vapors.

This lesion has been described in male rats of various strains, including Sprague-Dawley, Wistar, and Fischer 344. In addition to the nephropathy, Phillips reports a significant increase in urine volume and decrease in osmolality in Fischer 344 rats following exposure to Stoddard Solvent. Bruner and Pitts (1982) postulate that one mechanism which may contribute to the nephropathy is α-2μ microglobulin, a low molecular weight protein produced by the liver of male rats at puberty and readily filtered by the kidney. This protein has not been found in the livers of normal female rats. Bruner believes that this protein could be the major constituent of hyaline droplets, and factors resulting in its excessive accumulation in proximal tubular cells could contribute to the pathogenesis of hydrocarbon nephropathy.

This study was designed to determine the relative susceptibility to chronic nephropathy of four rat strains during and following inhalation exposure to 1000 mg/m^3 Shale derived JP-4 jet fuel. Urine metabolite analysis and protein measurement are made at selected times. These will be analyzed for correlation with the degree of chronic nephritis found in each rat species. The exposure conditions were selected to conform with previous fuel studies conducted in the Toxic Hazards Research Unit laboratory.

The Shale derived JP-4 sample used in this study is from the same source as that used in the Shale JP-4 90-day study previously described in this report and was subjected to the same quality control measures.

Four strains of male rats were exposed to 1000 mg/m^3 Shale JP-4 vapor on a continuous basis for 90 days. For these purposes, two Thomas Dome inhalation chambers were utilized. Chamber 5 contained 1000 mg/m^3 fuel while cham exposed controls were housed in Chamber 2. Each chamber housed 60 each of the following male rat strains: Fischer 344, Wistar, Sprague-Dawley, and Osborne Mendel.

Serial sacrifices of 10 rats of each strain took place at 45 exposure days and at the 90-day continuous exposure termination period. Additional interim sacrifices will occur at 6 and 12 months postexposure with all surviving rats sacrificed during the 24th month of the study. The order of sacrifice has been determined by a randomization list prepared by the UCI/THRU statistics department.

Ten rats of each strain, test and control, were sacrificed following 45 days of exposure. Blood and urine samples were collected and organ weights recorded at sacrifice. In addition to the routine blood values, methemoglobin was measured in five rats of each group.

The contaminant introduction and analysis systems for this study were identical to those described for the 90-day Shale JP-4 inhalation study described in this annual report.

Analysis of the vapor-air mixture resulted in a mean concentration within one tenth percent of the selected nominal value as shown in Table 38. No outward signs of toxic stress were observed in any strain and no exposure related deaths occurred during the 90-day exposure period.

TABLE 38. ANALYSIS OF SHALE JP-4 VAPOR CONCENTRATIONS INHALED BY FOUR STRAINS OF MALE RATS FOR 90 DAYS

Nominal Concentration, mg/m^3	1000
Mean Concentration, mg/m^3	1001
Standard Error	0.9
Lowest Daily Average, mg/m^3	959
Highest Daily Average, mg/m^3	1027

Mean body weights of the four strains are shown in Figures 18 through 21. The mean body weights of the 1000 mg/m^3 exposed rats parallel the mean weights of the control group but were slightly less during most of the study. No statistically significant differences between the mean body weights of the 1000 mg/m^3 exposed rats and those of the controls have been noted during the postexposure observation period.

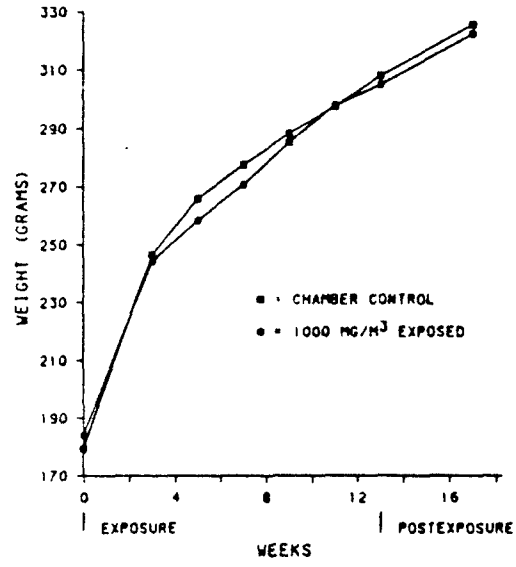

Figure 18. Mean body weights of male Fischer 344 rats continuously exposed to Shale JP-4 vapors for 90 days.

Urine osmolality values measured at each sacrifice period were compared with control values (Table 39.). None of the rat strains tested showed a statistically significant difference between the test and control values at 45 days. However, at 90 days all four strains showed a difference between test and control values which was statistically different in three strains but not in the fourth due to a wide range in measured values.

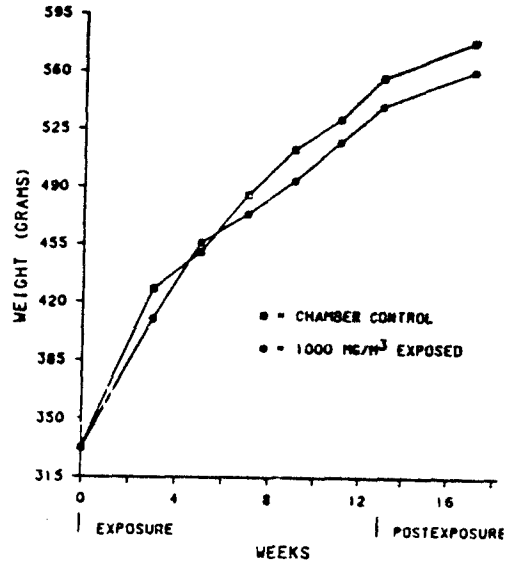

Figure 19. Mean body weights of male Sprague-Dawley rats continuously exposed to Shale JP-4 vapors for 90 days.

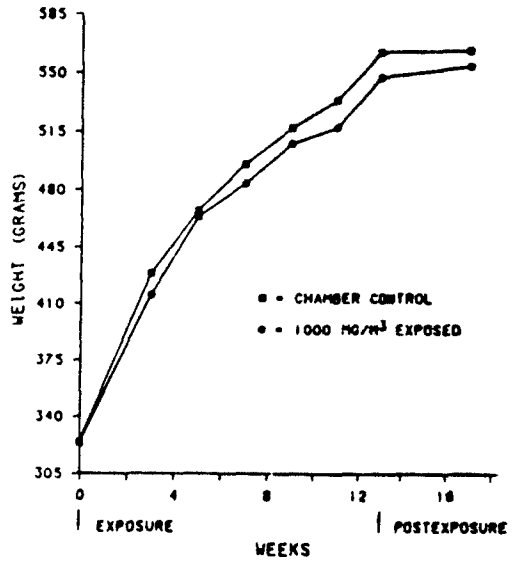

Figure 20. Mean body weights of male Wistar rats continuously exposed to Shale JP-4 vapors for 90 days.

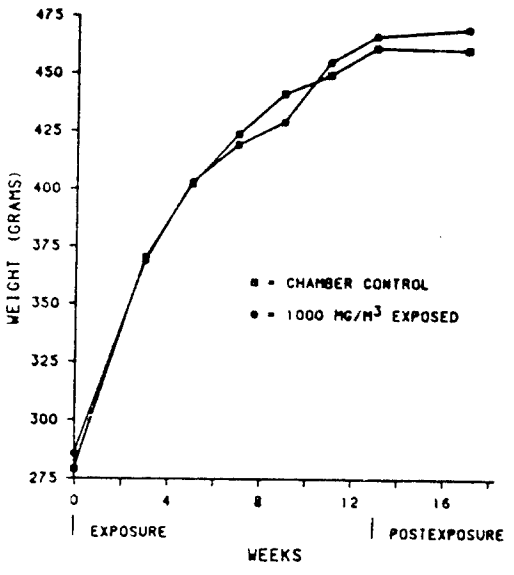

Figure 21. Mean body weights of male Osborne Mendel rats continuously exposed to Shale JP-4 vapors for 90 days.

TABLE 39. MEAN OSMOLALITY VALUES OF MALE RATS CONTINUOUSLY EXPOSED TO JP-4 SHALE VAPORS FOR 90 DAYS (N = 10)

Strain	Treatment	Osmolality[a] 0 days	45 days	90 days
F-344	Control	1640 ± 88	1146 ± 86	1728 ± 123
	1000 mg/m^3	1567 ± 80	826 ± 115	835 ± 81[c]
Sprague-Dawley	Control	1164 ± 124	785 ± 168	1148 ± 170[d]
	1000 mg/m^3	1287 ± 125	914 ± 120	670 ± 71[b]
Wistar	Control	1209 ± 163	776 ± 95	1222 ± 136
	1000 mg/m^3	1352 ± 164	1000 ± 101	877 ± 109
Osborne Mendel	Control	1426 ± 91	1330 ± 108	1215 ± 123
	1000 mg/m^3	1309 ± 193	1077 ± 82	837 ± 95[b]

[a] Mean ± SE (expressed as milliosmols/liter).
[b] Different from control value, $0.01 < p < 0.05$.
[c] Different from control value, $0.001 < p < 0.01$.
[d] N = 9.

At 45 days all strains of Shale JP-4 exposed rats showed a statistically significant change in urine pH values (Table 40) in the direction of urinary acidosis which could be indicative of renal disease or tubular dysfunction. However, by 90 days while all exposed rat values remained lower than contol values the difference was statistically significant only in the Sprague-Dawley strain.

TABLE 40. MEAN pH VALUES OF MALE RATS AFTER CONTINUOUS EXPOSURE TO JP-4 SHALE VAPORS (N = 10)

Strain	Treatment	pH Value[a] 0 days	45 days	90 days
F-344	Control	7.55 ± 0.15	7.65 ± 0.15	7.1 ± 0.2
	1000 mg/m^3	7.80 ± 0.13	7.00 ± 0.15[c]	6.8 ± 0.1
Sprague-Dawley	Control	8.35 ± 0.08	8.35 ± 0.08	8.1 ± 0.1[d]
	1000 mg/m^3	8.20 ± 0.11	7.55 ± 0.09[c]	7.6 ± 0.1[b]
Wistar	Control	7.45 ± 0.14	7.55 ± 0.16	7.5 ± 0.2
	1000 mg/m^3	7.20 ± 0.20	6.85 ± 0.13[c]	7.1 ± 0.2
Osborne Mendel	Control	6.95 ± 0.16	7.25 ± 0.24	7.5 ± 0.2
	1000 mg/m^3	7.35 ± 0.20	6.85 ± 0.13[c]	7.1 ± 0.2

[a] Mean ± SE.
[b] Different from control value, $0.01 < p < 0.05$.
[c] Different from control value, $0.001 < p < 0.01$.
[d] N = 9.

A number of hematology and clinical chemistry determinations made on male rats exposed to 1000 mg/m^3 were different from their respective controls at 45 days (Tables 41-44). The differences in hematology values occured primarily in the Fischer 344 rat strain. However, significant differences were seen in BUN and creatinine values of two of the four strains. No differences in methemoglobin values were found in any of the rat strains and this parameter was not measured in subsequent sacrifice groups.

Mean blood values from all strains of rats killed at exposure termination are summarized in Tables 45 through 48. Again, scattered differences are noted in the four strains but none of the values are outside normal ranges and no biological significance can be attributed to the noted values.

TABLE 41. FISCHER 344 RAT BLOOD PARAMETERS[a] AFTER EXPOSURE TO SHALE DERIVED JP-4 VAPORS FOR 45 DAYS

	Control	1000 mg/m^3
WBC (x10^3 cells/mm^3)	6.4 ± 0.3	7.9 ± 0.3
RBC (x10^6 cells/mm^3)	8.90 ± 0.09	8.32 ± 0.13
HGB (g/dl)	16.4 ± 0.2	16.1 ± 0.2
HCT (%)	45.4 ± 0.5	41.9 ± 0.6[b]
MCV (µm^3)	51.1 ± 0.2	50.4 ± 0.1[c]
MCH (pg)	18.5 ± 0.1	19.3 ± 0.1[b]
MCHC (g/dl)	36.2 ± 0.2	38.3 ± 0.2[c]
Glucose (mg/dl)	174 ± 12	132 ± 6[d]
BUN (mg/dl)	17.1 ± 0.3	20.9 ± 0.6[b]
Creatinine (mg/dl)	0.6 ± 0.03	0.8 ± 0.02[c]
Reticulocytes (%)	3.9 ± 0.4	4.3 ± 0.5

[a] Values expressed as mean ± SE, N = 9, 10.
[b] Statistically different from control at $p < 0.001$.
[c] Statistically different from control at $p < 0.01$.
[d] Statistically different from control at $p < 0.05$.

TABLE 42. SPRAGUE-DAWLEY RAT BLOOD PARAMETERS[a] AFTER EXPOSURE TO SHALE DERIVED JP-4 VAPORS FOR 45 DAYS

	Control	1000 mg/m^3
WBC (x10^3 cells/mm^3)	13.7 ± 0.7	12.5 ± 1.5
RBC (x10^6 cells/mm^3)	8.65 ± 0.19	8.20 ± 0.32
HGB (g/dl)	16.5 ± 0.3	16.1 ± 0.4
HCT (%)	46.1 ± 1.2	43.6 ± 1.5
MCV (µm^3)	53.3 ± 0.6	53.2 ± 0.7
MCH (pg)	19.1 ± 0.3	19.7 ± 0.5
MCHC (g/dl)	35.9 ± 0.5	37.0 ± 0.7
Glucose (mg/dl)	190 ± 12	156 ± 9
BUN (mg/dl)	18.0 ± 0.5	20.1 ± 0.8[b]
Creatinine (mg/dl)	0.6 ± 0.02	0.7 ± 0.04
Reticulocytes (%)	5.1 ± 0.7	4.4 ± 0.4

[a] Values expressed as mean ± SE, N = 9, 10.
[b] Statistically different from control at $p < 0.05$.

TABLE 43. WISTAR RAT BLOOD PARAMETERS[a] AFTER EXPOSURE TO SHALE DERIVED JP-4 VAPORS FOR 45 DAYS

	Control	1000 mg/m^3
WBC (x10^3 cells/mm^3)	9.2 ± 0.5	9.9 ± 0.8
RBC (x10^6 cells/mm^3)	8.40 ± 0.20	8.04 ± 0.21
HGB (g/dl)	16.7 ± 0.3	16.1 ± 0.3
HCT (%)	44.7 ± 1.2	42.6 ± 1.0
MCV (µm^3)	53.1 ± 0.4	53.0 ± 0.6
MCH (pg)	19.9 ± 0.4	20.2 ± 0.4
MCHC (g/dl)	37.6 ± 0.8	38.0 ± 0.5
Glucose (mg/dl)	177 ± 12	180 ± 13
BUN (mg/dl)	20.3 ± 0.5	21.4 ± 0.9
Creatinine (mg/dl)	0.6 ± 0.04	0.7 ± 0.03[b]
Reticulocytes (%)	4.7 ± 0.2	5.9 ± 0.4

[a] Values expressed as mean ± SE, N = 10.
[b] Statistically different from control at $p < 0.05$.

TABLE 44. OSBORNE MENDEL RAT BLOOD PARAMETERS[a] AFTER EXPOSURE TO SHALE DERIVED JP-4 VAPORS FOR 45 DAYS

	Control	1000 mg/m^3
WBC (x10^3 cells/mm^3)	6.9 ± 0.4	7.2 ± 0.6
RBC (x10^6 cells/mm^3)	8.97 ± 0.11	8.43 ± 0.31
HGB (g/dl)	17.7 ± 0.2	16.8 ± 0.5[b]
HCT (%)	47.7 ± 0.7	44.5 ± 1.5
MCV (µm^3)	53.2 ± 0.3	52.8 ± 0.3
MCH (pg)	19.8 ± 0.2	20.0 ± 0.3
MCHC (g/dl)	37.2 ± 0.3	37.9 ± 0.4
Glucose (mg/dl)	169 ± 15	128 ± 17[b]
BUN (mg/dl)	22.4 ± 0.7	23.7 ± 0.8
Creatinine (mg/dl)	0.7 ± 0.02	0.7 ± 0.03
Reticulocytes (%)	4.9 ± 0.4	5.1 ± 0.2

[a] Values expressed as mean ± SE, N = 9, 10.
[b] Statistically different from control at $p < 0.05$.

TABLE 45. FISCHER 344 RAT BLOOD PARAMETERS[a] AFTER EXPOSURE TO SHALE DERIVED JP-4 VAPORS FOR 90 DAYS

	Control	1000 mg/m^3
WBC (x10^3 cells/mm^3)	5.08 ± 0.06	7.03 ± 0.02[b]
RBC (x10^6 cells/mm^3)	8.59 ± 0.39	7.88 ± 0.11
HGB (g/dl)	15.06 ± 0.08	14.06 ± 0.02
HCT (%)	43.03 ± 2.06	39.07 ± 0.06
MCV (μm^3)	50.02 ± 0.08	50.3 ± 0.2
MCH (pg)	18.1 ± 0.2	18.6 ± 0.1[b]
MCHC (g/dl)	36.0 ± 0.4	36.9 ± 0.2
Glucose (mg/dl)	204 ± 15	177 ± 14
BUN (mg/dl)	16.9 ± 0.4	17.2 ± 0.4
Creatinine (mg/dl)	0.7 ± 0.05	0.8 ± 0.03
Reticulocytes (%)	3.6 ± 0.3	3.1 ± 0.4

[a] Values expressed as mean ± SE, N = 7, 10.
[b] Different from control value, $0.01 < p < 0.05$.

TABLE 46. SPRAGUE-DAWLEY RAT BLOOD PARAMETERS[a] AFTER EXPOSURE TO SHALE DERIVED JP-4 VAPORS FOR 90 DAYS

	Control	1000 mg/m^3
WBC (x10^3 cells/mm^3)	10.1 ± 1.2	11.8 ± 1.3
RBC (x10^6 cells/mm^3)	7.89 ± 0.19	7.60 ± 0.15
HGB (g/dl)	14.8 ± 0.2	14.2 ± 0.2
HCT (%)	40.9 ± 0.6	38.9 ± 0.9
MCV (μm^3)	52.0 ± 0.8	51.2 ± 0.4
MCH (pg)	18.8 ± 0.3	18.6 ± 0.2
MCHC (g/dl)	36.2 ± 0.3	36.4 ± 0.3
Glucose (mg/dl)	166 ± 9	179 ± 16
BUN (mg/dl)	14.8 ± 0.5	15.8 ± 0.6
Creatinine (mg/dl)	0.6 ± 0.03	0.7 ± 0.03
Reticulocytes (%)	4.9 ± 1.2	3.9 ± 0.4

[a] Values expressed as mean ± SE, N = 9, 10.

TABLE 47. WISTAR RAT BLOOD PARAMETERS[a] AFTER EXPOSURE TO SHALE DERIVED JP-4 VAPORS FOR 90 DAYS

	Control	1000 mg/m^3
WBC (x10^3 cells/mm^3)	7.3 ± 0.4	7.2 ± 0.5
RBC (x10^6 cells/mm^3)	8.23 ± 0.21	7.56 ± 0.12
HGB (g/dl)	14.7 ± 0.3	14.1 ± 0.1
HCT (%)	42.0 ± 1.0	39.9 ± 0.6
MCV (μm^3)	51.1 ± 0.3	51.4 ± 0.4
MCH (pg)	17.9 ± 0.2	18.2 ± 0.2
MCHC (g/dl)	35.0 ± 0.3	35.3 ± 0.3
Glucose (mg/dl)	206 ± 12	226 ± 15
BUN (mg/dl)	19.6 ± 1.0	20.6 ± 0.7
Creatinine (mg/dl)	0.6 ± 0.00	0.6 ± 0.02
Reticulocytes (%)	4.3 ± 0.5	4.3 ± 0.2

[a] Values expressed as mean ± SE, N = 10.

TABLE 48. OSBORNE MENDEL RAT BLOOD PARAMETERS[a] AFTER EXPOSURE TO SHALE DERIVED JP-4 VAPORS FOR 90 DAYS

	Control	1000 mg/m^3
WBC (x10^3 cells/mm^3)	5.9 ± 0.3	7.0 ± 0.3[b]
RBC (x10^6 cells/mm^3)	8.73 ± 0.21	8.02 ± 0.16
HGB (g/dl)	16.0 ± 0.4	14.6 ± 0.1[c]
HCT (%)	46.0 ± 1.4	42.5 ± 0.7[b]
MCV (μm^3)	52.6 ± 0.4	53.0 ± 0.3
MCH (pg)	18.4 ± 0.1	18.3 ± 0.3
MCHC (g/dl)	34.9 ± 0.3	34.5 ± 0.4
Glucose (mg/dl)	286 ± 39	256 ± 22
BUN (mg/dl)	18.8 ± 0.4	19.1 ± 0.9
Creatinine (mg/dl)	0.6 ± 0.03	0.7 ± 0.02
Reticulocytes (%)	3.6 ± 0.4	3.5 ± 0.2

[a] Values expressed as mean ± SE, N = 10.
[b] Different from control value, $0.01 < p < 0.05$.
[c] Different from control value, $0.001 < p < 0.01$.

Differences in BUN and creatinine values seen in two strains at 45 days were not noted in any strain at 90 days.

A summary of the organ weights of all rats sacrificed at 45 days is given in Table 49. No apparent adverse weight effects can be noted in the testis of any of the four strains. Statistically significant increases in mean liver weight were noted in three of the four strains while all strains showed a significant increase in mean kidney weights. This increase was particularly evident when comparing the kidney weights as a percentage of mean body weight.

TABLE 49. SUMMARY OF BODY AND ORGAN WEIGHTS OF FOUR RAT STRAINS EXPOSED TO SHALE DERIVED JP-4 VAPORS FOR 45 DAYS

Rat	Treatment	Mean Weight in Grams				% of Body Weight		
Strain	Group	Body	Testis	Liver	Kidney	Testis	Liver	Kidney
F-344	Control	258.0	2.905	7.54	1.818	1.128	2.924	0.706
F-344	1000 mg/m^3	246.9	2.943	8.49[b]	2.334[a]	1.193	3.439[b]	0.945[b]
S-D	Control	463.4	3.485	14.69	3.419	0.758	3.158	0.738
S-D	1000 mg/m^3	430.5[b]	3.422	13.84	4.547[b]	0.796	3.222	1.052[b]
Wistar	Control	470.5	3.512	13.72	2.940	0.750	2.907	0.626
Wistar	1000 mg/m^3	458.6	3.359	15.64[a]	3.818[b]	0.734	3.411[b]	0.832[b]
O M	Control	402.1	3.920	12.67	3.145	0.978	3.140	0.781
O M	1000 mg/m^3	399.0	3.979	14.83[b]	3.882[b]	0.999	3.713[b]	0.973[b]

[a] Significant at the 0.05 level.
[b] Significant at the 0.01 level.

The mean organ weights of all rats killed at 90 days are given in Table 50. A slight but significant increase in testis weight was seen in the Fischer 344 rats at 90 days that was not seen at 45 days. Statistically significant increases in mean liver weight were noted in two of the strains. At 45 days the Osborne Mendel rats showed an increase in gross liver weight but it was not apparent in those sacrificed at 90 days. All strains showed statistically significant increases in mean kidney weights as well as in the ratio of kidney weight to mean weight. This is the same as was noted in the four rat strains at 45 days.

This study is scheduled for termination in December 1985. Subsequent annual reports will contain additional experimental data as they become available.

TABLE 50. SUMMARY OF BODY AND ORGAN WEIGHTS OF FOUR RAT STRAINS EXPOSED TO SHALE DERIVED JP-4 VAPORS FOR 90 DAYS

Rat Strain	Treatment Group	Mean Weight in Grams				% of Body Weight		
		Body	Testis	Liver	Kidney	Testis	Liver	Kidney
F-344	Control	293.6	2.976	8.127	2.071	1.017	2.771	0.706
F-344	1000 mg/m^3	285.3	3.067[a]	9.324[b]	2.613[b]	1.076[a]	3.268[b]	0.916[b]
S-D	Control	537.2	3.612	15.688	3.806	0.676	2.913	0.711
S-D	1000 mg/m^3	527.2	3.399	17.561	4.885[b]	0.649	3.334[b]	0.927[b]
Wistar	Control	531.1	3.326	15.358	3.288	0.628	2.886	0.618
Wistar	1000 mg/m^3	533.2	3.479	17.929[a]	4.276[b]	0.660	3.354[b]	0.814[b]
O M	Control	440.9	4.166	14.894	3.407	0.948	3.376	0.772
O M	1000 mg/m^3	447.7	4.007	15.943	4.264[b]	0.897	3.567	0.954[b]

[a] Significant at the 0.05 level.
[b] Significant at the 0.01 level.

TOXICITY OF O-ETHYL-O'-(2-DIISOPROPYLAMINOETHYL)METHYL-PHOSPHONITE (EDMP)

During 1982, a series of experiments were planned and initiated to characterize the acute toxicity of EDMP. Previous acute exposures by the inhalation and IV routes (Dimmick, Jr., et al., 1979) had indicated that EDMP was moderately toxic. Subchronic exposures of 27 weeks had also been conducted at concentrations of 3 and 15 mg/m^3 using Sprague-Dawley/Wistar rats, ICR Swiss and "A" strain mice, and Hartley guinea pigs. The only positive finding was depression of red blood cell cholinesterase at exposure termination in rats exposed to both concentrations and in mice exposed to the higher level. Because previous studies did not have adequate characterization of the chemical nature of the material delivered to the test animals, the THRU was requested to plan and carry out a new series of acute and subchronic experiments as shown in Table 51.

Before inhalation exposures began, EDMP aerosols were generated into test chambers for measurement of decomposition rates and determination of degradation products under varying

conditions of relative humidity, in air or nitrogen to characterize the important factors contributing to EDMP breakdown in inhalation chambers.

TABLE 51. SCHEDULED ACUTE AND SUBCHRONIC TOXICITY EXPERIMENTS ON EDMP

Acute Experiments

Oral Toxicity -	Sprague-Dawley rats and ICR Swiss mice, male and female
Intraperitoneal Toxicity -	Sprague-Dawley rats and ICR Swiss mice, male and female
Dermal Toxicity -	New Zealand albino rabbits, male and female
Skin Sensitization -	Hartley albino guinea pigs, female
Skin Irritation -	New Zealand albino rabbits
Eye Irritation -	New Zealand albino rabbits
Delayed Neurotoxicity -	Leghorn hens
Inhalation Toxicity -	Fischer 344 rats and B6C3F1 mice, male and female
Urinary Metabolites -	Fischer 344 rats, male and female

Subchronic Experiment

13-Week Inhalation -	Fischer 344 rats and B6C3F1 mice, male and female

The results of the decomposition experiments and all acute studies except inhalation and neuropathology were reported in last year's annual report (MacEwen and Vernot, 1983). The interpretations of micropathological examination of neural tissue from hens exposed to EDMP became available during 1983, and acute inhalation exposures were completed in time for inclusion in this annual report.

Micropathology of Neural Tissue from Hens Administered EDMP

The progression of neurotoxic signs in hens following EDMP administration was different than in birds given triorthocresylphosphate (MacEwen and Vernot, 1983), and these differences had their counterpart in the microscopic lesions noted in nerve

tissue. The effects were similar in animals given single or multiple intermittent doses, so only the pathology in single-dosed animals will be described.

The hens exposed to TOCP exhibited the classic lesions of delayed neuropathy (Smith and Lillie, 1931; Barnes and Denz, 1953; Cavanagh, 1954; and Fenton, 1955). These lesions included: (1) segmental swelling of axons to form amorphous, globular enlargements; (2) vacuolization and/or fragmentation of axonal segments; and (3) axonal lysis. Accompanying the axonal change was a ballooning and degeneration of the associated myelin sheath.

In contrast, the lesion seen in animals given EDMP was principally associated with myelin and was distinguished from similar findings in negative control animals only by the higher frequency in exposed hens. A segmental disruption of the myelin sheath of nerve fibers was observed in both the spinal cord and/or the sciatic nerve of 86% of the hens exposed to EDMP. Similar lesions were also noted in 30% of the corn oil controls, although with somewhat less frequency in each section. This lesion was characterized by a slight ballooning of the myelin sheath which contained a clump of degenerating myelin. The lesion was almost always multifocal in distribution and minimal in severity. Kluver-Barrera stains demonstrated the lesion to be confined to the myelin sheath with little apparent effect on the axon.

The lesions noted in nerve tissue sections from all treatment groups are listed for comparison in Table 52, and photomicrographs of lesions typically found in EDMP and TOCP treated hens are shown in Figures 22 and 23. In the photomicrographs of tissue from EDMP-treated hens, the arrows identify areas of segmented myelin swelling. Regions of the myelin sheath are ballooned, in some cases appearing like "soap bubbles", and usually contain a clump of degenerative material within the ballooned portion. The axons, visible as dark linear segments in the Kluver-Barrera stains, are usually pushed aside and appear relatively unaffected.

The photomicrographs of tissue from TOCP-treated hens demonstrate degenerating nerve fibers. Segmented swelling (arrows) of the axons is obvious. The segments are amorphous in appearance and the myelin sheath is ballooned and fragmented.

TABLE 52. GRADED LESIONS IN NEURAL TISSUE AFTER CORN OIL, TOCP, AND EDMP SINGLE-DOSE ADMINISTRATION[a]

Corn Oil Controls					TOCP, 500 mg/kg					EDMP, 589 mg/kg				
Hen No.	C[b]	T[c]	L[d]	S[e]	Hen No.	C[b]	T[c]	L[d]	S[e]	Hen No.	C[b]	T[c]	L[d]	S[e]
10	NL	B1	B1	NL	09	E3	B2	B1	B1	08	NL	NL	NL	NL
15	NL	NL	NL	NL	12	E3	B2	B1	B1	11	NL	NL	B1	B1
25	NL	NL	NL	NL	24	B2	B1	C2	NL	19	B1	B1	NL	B1
28	NL	NL	NL	NL	27	B2	E3	B1	NL	21	B1	B1	NP	NP
35	NL	NL	NL	NL	46	NL	NL	NL	B2	31	B1	B1	B2	NL
38	NL	NL	NL	NL	54	C3	B2	B2	B1	33	B1	B1	B1	A1
43	NL	B1	B1	NL	56	E3	B2	NP	B2	36	NL	B1	B1	A1
44	B1	B1	NL	NL	57	D3	D2	B1	NL	45	B1	B1	B2	B2
48	NL	NL	NL	NL	60	B2	B2	B1	NL	50	NL	NL	NL	NL
69	NL	NL	NL	NL	65	B2	B1	C2	NL	55	NL	B1	NL	NL
										59	B1	B2	NL	B1
										61	B1	B1	B1	B1
										63	B1	B1	B1	NL
										66	NL	B1	B1	NL

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a All Sections are Longitudinal
b Cervical Spinal Cord
c Thoracic Spinal Cord
d Lumbar Spinal Cord
e Sciatic Nerve

NL - No Lesion
NP - Tissue Not Present
A - Focal
B - Multifocal
C - Patchy
D - Segmental
E - Zonal

1 - Minimal (No Axonal Involvement)
2 - Mild
3 - Moderate

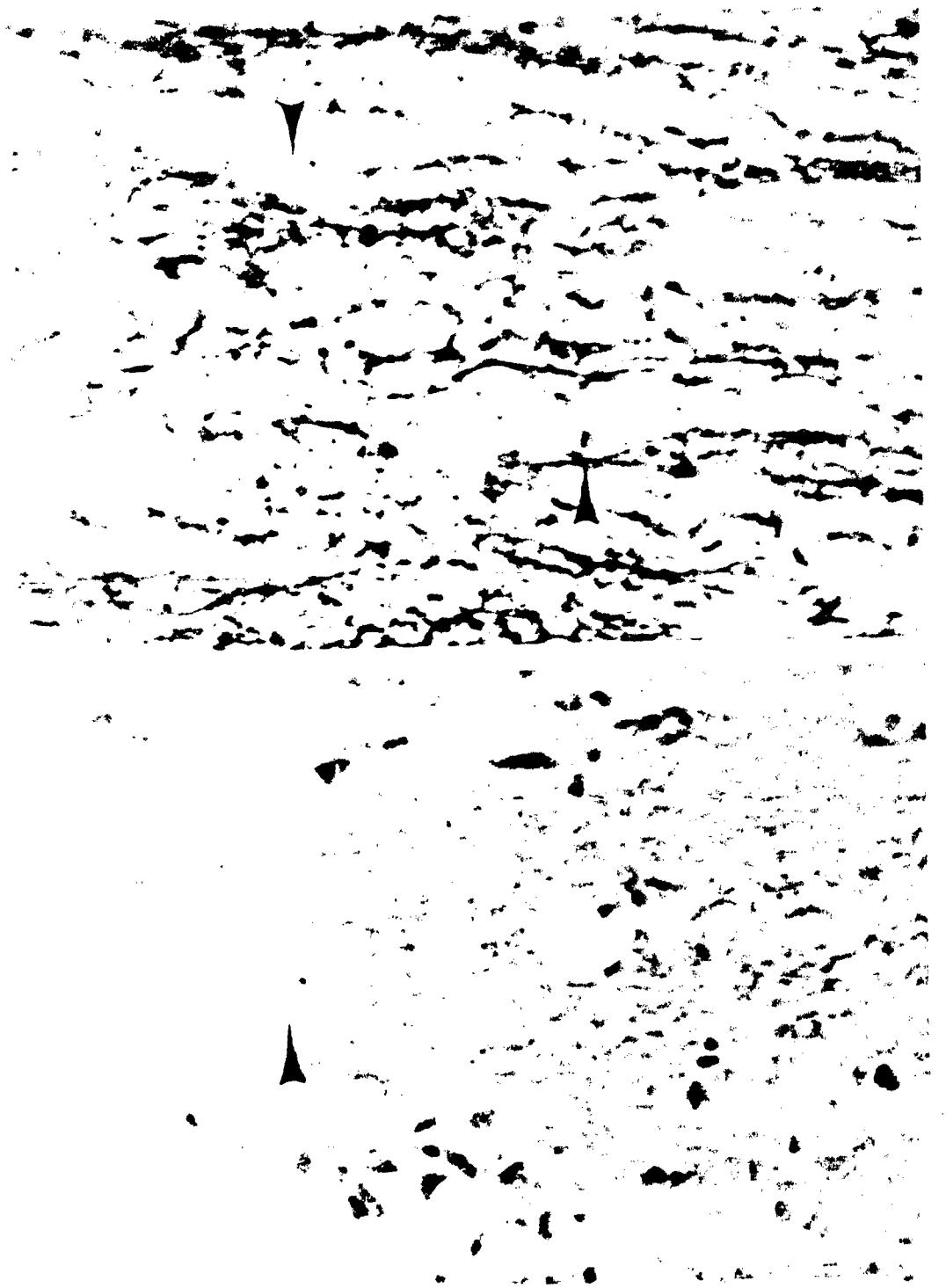


Figure 22. Longitudinal sections of spinal cord of EDMP-treated hen showing myelin degeneration (arrows), 500 x, H & E - bottom, Kluver-Barrera - top.

Figure 23. Longitudinal section of spinal cord of TOCP-treated hen showing axonal degeneration (arrows), 500x, H & E - bottom, Kluver-Barrer - top.

Inhalation Toxicity of EDMP to Rats and Mice

During exposure, two kinds of analyses were performed: 1) samples of chamber air were passed through ethyl ether in midget impingers and the ether injected into a gas chromatograph to determine the distribution of EDMP and decomposition products; 2) chamber atmosphere was absorbed continuously in 4-methylcyclohexanol and total concentration of EDMP-derived compounds determined by infrared absorption. The development of these methods is detailed in the Facilities Section of this report. Figure 24 is a typical GC of an exposure chamber sample. This distribution of all the peaks varied somewhat during the exposures and from one exposure to the other, but the changes did not seem to be associated with significant mortality effects.

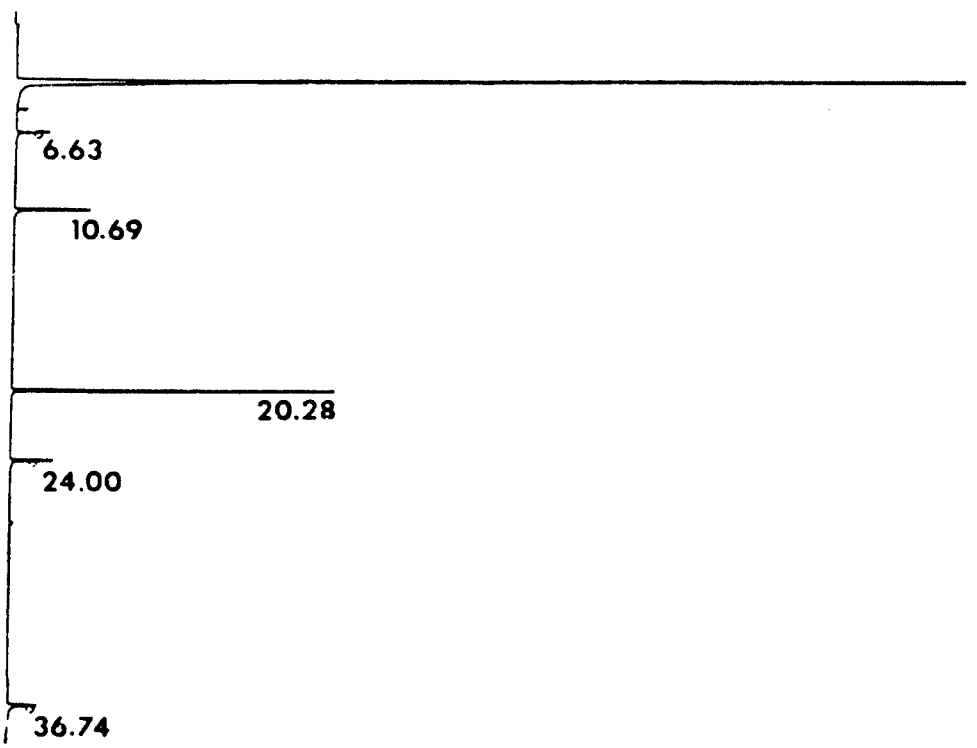


Figure 24. Gas chromatogram of sample from exposure chamber at concentration of 1930 mg/m^3 EDMP. Peak retention times are printed on chromatogram.

Table 53 is a listing of mortality in rats and mice resulting from 6-hour exposures to various concentrations of EDMP aerosol. Also listed are particle diameters and mean relative EDMP contents of the aerosols. The precisions of these LC_{50} determinations are not as good as those expected from stable compounds, probably reflecting the variation in distribution of the decomposition products from exposure to exposure.

TABLE 53. ACUTE 6-HOUR INHALATION TOXICITY OF EDMP TO FISCHER 344 RATS AND B6C3F1 MICE

Conc. mg/m^3	S.D. mg/m^3	Rats Male	Rats Female	Mice Male	Mice Female	Particle Diam µm	% EDMP
4866	430			10/10		4.1	56
3944	196	10/10	10/10	10/10	10/10	4.2	51
3786	305			7/10		3.9	54
3500	422			2/10		3.6	40
3357	334			1/10		3.1	42
3012	399			1/09		3.6	47
2992	165			0/10		3.6	53
2695	498			3/10		3.5	32
2676	206	7/10	10/10	4/10	8/10	3.6	45
2532[a]	429	4/10				3.0	77
2276	198	2/10		0/10	5/10	3.2	51
1989[a]	105	1/10		0/10		3.7	63
1931	219	2/10	10/10	0/10	0/10	3.3	59
1301	88	3/10	10/10			3.0	58
950[a]	274	1/10				3.6	45
778[b]	150		10/10			3.3	42
544[a]	68	0/10				3.7	47
527[b]	62		6/10			2.5	72
226[a]	35	0/10	6/10			2.1	30
115[a]	16		2/10			1.6	44
72[a]	17		4/10			1.8	27
57	15		1/10			1.8	33
50[a]	18		0/10			2.1	27
LC_{50}		2520	209	3502	2360		
95% C.L.		(2130-3280)	(142-339)	(3036-4877)	(2179-2567)		

[a] Second drum of EDMP.
[b] Experiments run later (3 months) to confirm LC_{50} value for female rats.

In calculating the LC_{50} values for male and female Fischer 344 rats, the results of two exposures were not used, since they lay so far out of the pattern for males given by all other exposures. The LC_{50} values indicate that there is not much difference among male rats and both sexes of mice in the lethal effects of acute exposure, but that female rats are much more susceptible, with an LC_{50} an order of magnitude lower. Toxic signs during and following acute exposures were consistent with prolonged cholinergic activation including diarrhea, exophthalmos, fine tremors which could persist for 48 hours, and chromodacryorrhea appearing after 24 hours.

There is a rough inverse correlation between chamber concentration and both particle size and EDMP content of the aerosol. This is not surprising, since vaporization rates might be expected to be higher from low concentration aerosols. This would lead to smaller particle sizes and higher vapor concentration relative to the aerosol. If decomposition takes place faster in the vapor state, which is probable, EDMP content would be smaller at low concentrations.

RAT URINARY METABOLITES OF EDMP

The total ion chromatogram obtained from GC/MS analysis of a concentrated ether extract of male rat urine after IP administration of 0.25 ml/kg EDMP is presented in Figure 25. The largest peak at 20 minutes RT is due to diisopropylaminoethanol, one of the hydrolysis products of EDMP. The other peaks in the GC are of compounds normally found in rat urine. Subsequent hydrolysis of the urine using β-glucuronidase and HCl did not reveal any compounds other than normal urine constituents. The other hydrolysis product, O-ethylmethylphosphinate, was not detectable in the GC, and the possibility existed that it was hidden in the solvent peak or that it eluted early or late at a point where it was not visible. In order to investigate this possibility, 60 µl of EDMP was added to 10 ml of water and allowed to stand for 24 hours to ensure complete hydrolysis. Three ml was then extracted with ether in the same manner as urine. The total ion chromatogram obtained on injection of the concentrated ether extract is given in Figure 26. In this GC, O-ethylmethylphosphinate elutes at 22 minutes where it would be easily identified in the chromatogram of rat urine. It appears that O-ethylmethylphosphinate is not excreted in the urine after exposure of rats to EDMP and the only detectable decomposition product is diisopropylaminoethanol. The GC pattern of female rats given IP doses of EDMP

was the same as that of males. No urinary compounds derived from EDMP were detected in the urine of male or female rats exposed to 2300 mg/m^3 for 6 hours.

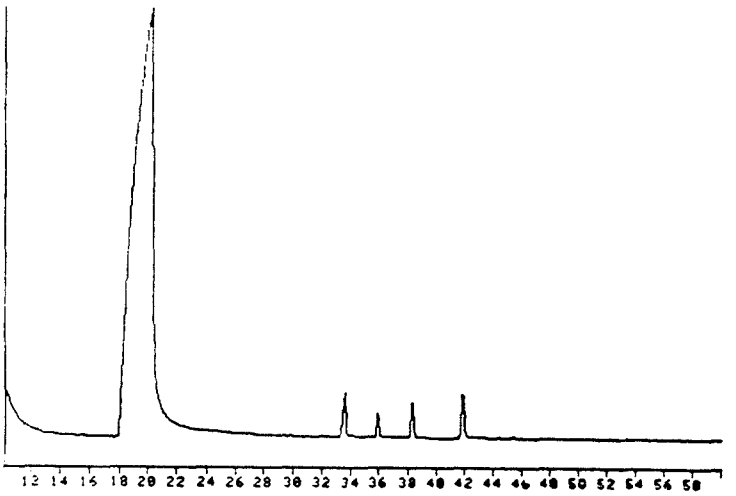
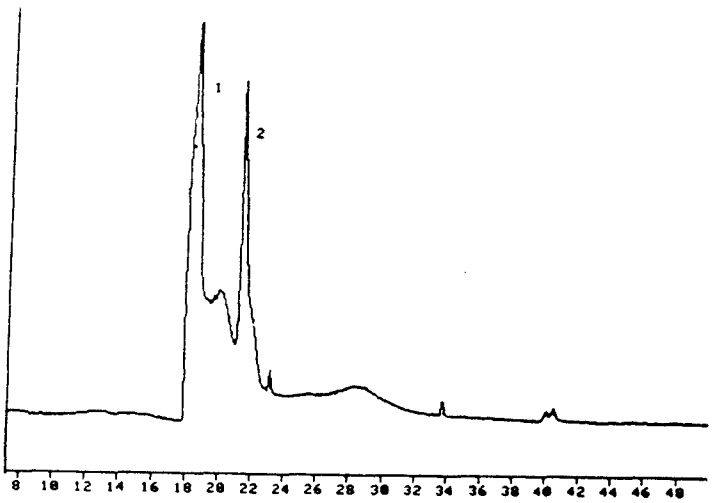


Figure 25. Total ion chromatogram of concentrated ether extract of urine from male rats dosed IP with EDMP.



Retention time, min.

1. Diisopropylaminoethanol
2. O-Ethylmethylphosphinate

Figure 26. Total ion chromatogram of concentrated ether extract of aqueous solution of EDMP held for 24 hours.

SUBCHRONIC 90-DAY CONTINUOUS INHALATION EXPOSURE TO DIMETHYL METHYLPHOSPHONATE

Dimethyl methylphosphonate (DMMP) has been used as a nerve gas simulant by the military. A possibility for human exposure exists during experimental testing and to evaluate the need for emergency exposure limits or Threshold Limit Values (TLV's) the THRU was asked to conduct a 90-day subchronic inhalation toxicity study.

Hollingshaus et al. (1981) reported that 10 daily repeated intraperitoneal injections of DMMP at 50 mg/kg failed to produce signs of delayed neurotoxicity in adult white Leghorn hens.

Dunnick et al. (1984) investigated the reproductive toxicity of DMMP. Male rats were gavaged with DMMP at doses up to 2000 mg/kg for a 90-day period. Dose related decreases in sperm count, sperm motility, and male fertility index resulted with an increased number of resorptions occurring in females mated to exposed males. While DMMP altered reproductive capability at all levels tested, only the 2000 mg/kg treatment level produced any microscopic lesions in testes. These changes included lack of spermatogenesis or degeneration, vacuolization and necrosis of spermatogenic cells.

A subchronic study with Fischer 344 rats gavaged 5 days per week for 13 weeks indicated no significant signs of toxicity or changes in body weight at doses up to 1000 mg/kg (LBI project No. 10608-13 final report). All 20 rats dosed at 4000 mg/kg died within the first week of dosing. Eight of 19 rats dosed at 2000 mg/kg died during the study with both sexes of rats showing increased liver weight. Microscopic examination revealed lesions in kidneys, testes, and salivary glands which may have been related to DMMP exposure. The report indicated that DMMP exacerbated male rat spontaneous nephropathy and may have reduced the life span of male rats with prolonged administration. The significance of the salivary gland lesions was questionable due to the presence of sialodacryoadenitis virus infection which may have been activated by the DMMP.

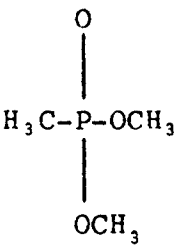
Because inhalation of DMMP vapor is expected to be a prime exposure route, inhalation toxicity tests were requested by the Air Force. Prior to initiation of the 90-day study, laboratory animals were exposed to DMMP at a saturated vapor concentration to determine the acute inhalation hazard. To aid in setting concentrations for the 90-day exposure, 2-week pilot studies were also conducted.

Materials and Methods

DMMP was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin and was received in 15 gallon, polyethylene lined drums. A Varian 3700 gas chromatograph was used for quality control analysis of all samples of DMMP obtained. This analysis was repeated when each drum was installed in the generation system in order to test the stability of the DMMP in storage. All drums were found to contain identical material with no decomposition of the DMMP during storage. The properties of DMMP are listed in Table 54.

TABLE 54. PROPERTIES OF DIMETHYL METHYLPHOSPHONATE (DMMP)

Formula:



CAS Reg. No.:	756-79-6
Molecular Weight:	124.08
Chemical State (20°C):	liquid
Liquid Density (g/cc):	$d\ \frac{20}{4}\ 1.145$
Vapor Density (g/cc): (compared to air):	4.3
Boiling Point (°C):	181°
Flash Point (°C):	43°[a]
Vapor Pressure (mm Hg at 20°C):	0.61
Volatility (mg/m^3):	4100
Viscosity (cp at 20°C):	4.1

[a] Discrepancies exist in the literature on DMMP flash point. UCI/THRU found a flash point of 115°C by the open cup method.

Saturated Vapor Test - Concentrated vapors of DMMP were generated in a gas wash bottle equipped with a fritted glass disc. The bottle contained a predetermined amount of DMMP. Dried air was blown through the bottle at a known rate. The resulting air-vapor mixture was conducted to a 60 liter plastic chamber containing 5 male Fischer 344 rats (200-300 g) and 5 male B6C3F1 mice (20-25 g). Analytical concentrations were not measured; however, estimated concentrations were calculated by material balance measurements. Exposure lasted for 6 hours. All animals were observed frequently during the exposure and twice daily during a 14-day holding period. Visible signs of toxicity were recorded and body weights were measured on days 0, 1, 4, 7, 10, and 14. Food and water were available ad libitum except during exposure.

2-Week Inhalation - DMMP vapors were generated by passing liquid DMMP through a heated glass evaporator column. Air passing through the column was directed into a 2 m^3 Rochester chamber containing 10 male and 10 female Fischer 344 rats and 10 male and 10 female B6C3F1 mice. An equal number of animals serving as a sham exposed control group was housed in a second Rochester chamber.

Analytical concentrations were continuously monitored with an infrared analyzer set at a wave length of 8.4 μm. Exposures were run continuously for 14 days. The generation system was shut down for less than 1 hour each day for animal care and weighing as scheduled. The animals were observed hourly for visible signs of toxicity. Body weights were measured on days 0, 3, 7, 10, exposure termination, and one week postexposure. The animals had food and water ad libitum during the study.

90-Day Inhalation - Mice and rats were exposed to DMMP vapor on a continuous basis for 90 days. For these purposes, Thomas Dome inhalation chambers were utilized with two DMMP exposure concentrations tested. Sham exposed controls were also maintained. Each chamber originally housed 85 male and 85 female Fischer 344 rats and 100 male and 100 female C57BL/6 mice.

Following the 90-day continuous exposure period, 15 rats and 25 mice of each sex from each group were killed for evaluation of gross tissue injury and histologic examination. Additional interim sacrifices of ten rats and mice of each sex and group were scheduled for 3 and 12 months postexposure. The study will be terminated after 24 months and the remaining animals will be killed for pathologic examination.

All animals were routinely observed during the exposure and postexposure periods. Rats were individually weighed at biweekly intervals during exposure and are presently weighed monthly during postexposure. Mice are weighed monthly by cage groups.

The blood tests listed in Table 55 were performed on all rats killed at exposure termination and 3 months postexposure. These tests will also be performed on rats killed 12 months post-exposure and on 10 male and 10 female rats from each group at the end of the study.

TABLE 55. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON RATS EXPOSED TO DMMP VAPORS

Hematology	Chemistry
Hematocrit	Calcium
Hemoglobin	Albumin/Globulin
Total RBC	Total Protein
Total WBC	Glucose
Differential	Alkaline Phosphatase
Mean Corpuscular Volume (MCV)	SGPT
Mean Corpuscular Hemoglobin (MCH)	SGOT
Mean Corpuscular Hemoglobin Concentration (MCHC)	Bilirubin
	Creatinine
	BUN

Whole body, testes, liver, kidney, and spleen weights were obtained from all rats killed at exposure termination and 3 months postexposure. These organ weights will also be observed at 12 months postexposure, and on 10 male and 10 female rats from each group at the end of the study.

DMMP vapor was generated by a pair of electrically heated, glass evaporator towers operating in parallel. A schematic diagram of the DMMP generation system is presented in Figure 27. A metered flow of liquid contaminant was introduced at the top of each tower into a countercurrent stream of air. The combined contaminant/air outputs of each tower were transported in 3/4" I.D. stainless-steel tubing which was subsequently partitioned by an adjustable splitter valve for introduction into the exposure chambers. Airstream and liquid effluent temperatures were monitored by probes placed at the top and bottom of the towers. Temperature monitors were also components of an automated alarm and emergency shutdown system in case of overheating during unattended operation.

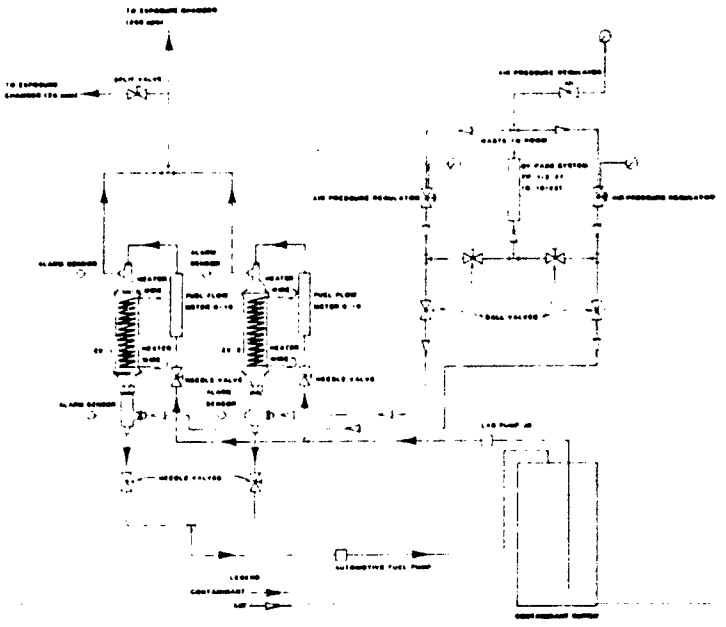


Figure 27. Chamber introduction system for DMMP inhalation exposure vapor generation.

Liquid DMMP was pumped through teflon transmission tubing from a supply drum to the evaporator towers by a variable flow, ceramic-piston pump. An automotive fuel pump transported tower effluents of unvaporized DMMP back to the supply drum. Less than fifty percent of the DMMP was vaporized in one pass of the liquid through the generator. Therefore, generator effluent was recycled, rather than wasted, in order to conserve the supply. Since the contaminant was essentially a pure substance, as opposed to a mixture, no significant compositional changes in the generator supply due to fractionation were expected to result from recycling the DMMP.

After about 70 days of continuous operation the ceramic-piston pump malfunctioned due to deterioration of the pump's Kynar® seals probably caused by the DMMP. With minor modification a new liquid delivery system was installed which used one of the automobile fuel pumps. The neoprene seals of these pumps were known to be even more susceptible to decomposition by DMMP than the seals of the other type of pump. The automobile fuel pumps were, however, relatively inexpensive and easily obtained through auto-supply retailers and were used in the generation system for the remainder of the exposure.

Chamber concentration of DMMP vapor was continuously monitored by the infrared analyzer equipped with a 20 meter gas cell. Instrumental analog output was referenced to percent full-scale displacement on a strip-chart recorder. The three-way electromechanical valve system controlled by the Transwave analytical timer and alarm system was used to provide alternating, ten minute readings of chamber atmosphere and chamber input air, the latter serving as a baseline reference.

The chamber sampling site was at the height of the third of five cage tiers and at a distance of about one foot from the chamber window. Instrumental settings of the infrared analyzers monitoring each chamber are given in Table 56.

TABLE 56. INFRARED ANALYZER SETTINGS FOR DMMP VAPOR ANALYSIS

Miran 1A Settings	Exposure Concentration 25 ppm	250 ppm
Wavelength (μm)	8.4	8.4
Slit Width (mm)	1	1
Optical Path Length (m)	14.25	12.75
Response	1	1
Absorbance Range (AU)	0 - 0.25	0 - 1
Chart Recorder Amplification (volts per full-scale)	1	1

Atmospheres of both high level exposure chambers were analyzed by gas chromatography twice during the exposure to insure that no decomposition of the DMMP had occurred during vapor generation. Samples were obtained by bubbling chamber atmosphere through 20 ml of diethyl ether in a midget-impinger. The samples were drawn for twenty minutes at a flow rate of 2.5 L per minute. During the sampling interval the ether volume was reduced to about two milliliters. The DMMP ether solution was analyzed with a Varian 3700 gas chromatograph.

The exposure chamber atmospheres were routinely analyzed for the presence of vapor condensate aerosol with a Royco® optical particle counter.

Results

Saturated Vapor Test - The nominal concentration for the single 6-hour exposure was calculated to be 3300 mg/m^3. The theoretical saturated vapor concentration for DMMP is 4100 mg/m^3. No deaths resulted from the 6-hour exposure to 3300 mg/m^3 but weight loss was noted in exposed rats 1 week postexposure (Table 57). At 2 weeks postexposure the exposed rats had shown some weight gain, but the mean weight of the group remained less than unexposed controls. Mouse body weights appeared unaffected by exposure.

TABLE 57. MEAN BODY WEIGHTS (GRAMS) OF MALE RODENTS EXPOSED TO 3300 mg/m^3 DMMP FOR 6 HOURS

	Rat (N = 5)		Mouse (N = 5)	
	Control	Exposed	Control	Exposed
Preexposure	210	207	21	21
1 week postexposure	217	198	22	23
2 week postexposure	233	226	26	26

Because the single exposure to a near saturated vapor concentration failed to produce mortality, repeated 4-hour exposures to saturated vapors were conducted. Five male Fischer 344 rats and 5 male B6C3F1 mice were exposed to DMMP saturated vapors 9 times over a 2-week period. Exposures were conducted on weekdays only. The mean nominal concentration for the 9 exposures was 3893 mg/m^3 with a standard deviation of 278 mg/m^3. No deaths resulted from this series of exposures. Rat body weights measured during the course of the exposures showed a 12-gram gain between the first and last exposure, a 12-day period. This represents an average daily gain of 1 gram/day. During the 6 days following the last exposure the rats gained 14 grams, greater than 2 grams/day. Mouse body weights did not appear to be affected by DMMP exposure.

2 Week Inhalation - A target concentration of 500 ppm DMMP was chosen for the 2-week continuous inhalation test. The actual analyzed concentration mean for the 14-day exposure was 478 ppm with a standard deviation of 27 ppm.

After 3 days of exposure the rats were lethargic. Porphyrin tinged nasal discharge was evident in most of the male rats. By day 6 of the exposure the male rats were openly aggressive when

disturbed. Nasal discharge continued in male rats and began to appear in the female rats. One male rat was removed from the exposure in a moribund condition with what appeared to be bite wounds on the neck area. Two male mice were dead by day 6. On day 7 fresh nasal discharge was noted in female rats. Nasal discharge subsided during subsequent exposure days, but mild lethargy continued.

Both sexes of rodents exposed to 500 ppm DMMP lost a substantial amount of body weight after 3 days of exposure (Table 58). General weight loss continued in most of the animals to the end of the exposure but was less rapid during the latter half of the exposure period.

TABLE 58. BODY WEIGHTS[a] OF RODENTS EXPOSED TO 500 PPM DMMP CONTINUOUSLY FOR 2 WEEKS

	Exposure Day				
	0	3	7	10	14
Male Rat					
Control	204 (10)	212 (10)	217 (10)	220 (10)	228 (10)[b]
500 ppm	202 (10)	178 (10)	164 (9)	160 (8)	164 (8)
Female Rat					
Control	150 (10)	150 (10)	152 (10)	150 (10)	152 (10)[b]
500 ppm	146 (10)	133 (10)	127 (10)	129 (9)	119 (9)
Male Mice					
Control	21 (10)	22 (10)	22 (10)	22 (10)	23 (10)
500 ppm	22 (10)	21 (10)	22 (8)	20 (7)	19 (6)
Female Mice					
Control	18 (10)	18 (10)	18 (10)	19 (10)	20 (10)
500 ppm	19 (10)	17 (10)	16 (10)	13 (5)	14 (2)

[a] Mean body weight, grams (N).
[b] Weighed on Day 13.

Significant mortality occurred in the animals exposed to 500 ppm DMMP for 2 weeks (Table 59). No mortality occurred in the rodents serving as controls.

TABLE 59. CUMULATIVE MORTALITY OF RODENTS EXPOSED TO 500 PPM DMMP CONTINUOUSLY FOR 2 WEEKS

	Exposure Day														Mortality Ratio[a]
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Male Rats							1[b]				2					2/10
Female Rats									1							1/10
Male Mice							2		3					4		4/10
Female Mice									2	4	5	6	8			8/10

[a] Total number dead/initial group size.
[b] Moribund rat removed.

The original protocol for the 2-week study called for urine collection from the control and exposed rats for metabolite identification. At the conclusion of the collection period all animals, control and exposed, were to be killed for gross examination. Because of the number of deaths occurring in the DMMP exposed animals it was decided to repeat the 2-week exposure at a lower concentration. To eliminate the delay associated with ordering new animals we decided to use the controls from the original study as the exposure group for the repeat study. Therefore, only the exposed rodents were killed at the conclusion of the urine collection period at which time blood was collected from four male and five female DMMP exposed rats for analysis. The results of these tests are shown in Table 60. Since blood samples from the controls were not obtained for hematological analysis, the values of historical controls from the THRU data base are shown for comparison. Although there were slight reductions in blood cell counts and hematocrit levels, the rats exposed to DMMP did not display any remarkable alterations from normal hematology values.

Orbital blood samples were obtained from control and exposed rats for measurement of cholinesterase activity at the conclusion of the exposure. The results of these tests are shown in Table 61. Exposure to 500 ppm DMMP produced a mild depression in erythrocyte cholinesterase activity.

The results obtained during the 2-week exposure to 500 ppm DMMP clearly indicated that this concentration was too high for continuous exposure for 90 days. The exposure concentration was

lowered to 300 ppm and a second 2-week exposure was begun with the rats (10 male and 10 female) and mice (10 male and 10 female) used as controls in the 500 ppm study. No separate control group was available for comparison in this study.

TABLE 60. EFFECT OF 2 WEEKS OF EXPOSURE TO 500 PPM DMMP ON RAT HEMATOLOGY VALUES[a]

	Historical Control		500 ppm DMMP	
	Male (N=362)	Female (N=354)	Male (N=4)	Female (N=5)
RBC ($x10^6$ cells/mm^3)	8.67 ± 0.1	7.81 ± 0.1	7.44 ± 0.33	7.10 ± 0.17
WBC ($x10^3$ cells/mm^3)	5.9 ± 0.1	5.1 ± 0.2	4.8 ± 0.4	5.4 ± 0.5
HCT (%)	47.3 ± 0.3	43.3 ± 0.2	41.8 ± 1.3	37.8 ± 0.5
HGB (g/dl)	15.7 ± 0.1	14.6 ± 0.1	14.4 ± 0.5	14.2 ± 0.3
MCV (cu microns)	55.1 ± 0.4	56.0 ± 0.3	56.1 ± 0.8	53.2 ± 1.0
MCH (micro-micrograms)	18.3 ± 0.1	18.9 ± 0.1	19.3 ± 0.2	20.0 ± 0.1
MCHC (%)	33.3 ± 0.1	33.8 ± 0.1	34.5 ± 0.3	37.6 ± 0.5

[a] Mean ± SE.

Male rats showed a weight loss during the first days of the exposure to 300 ppm DMMP (Table 62). This was followed by a slow gradual weight gain. Female rats and mice of either sex failed to show any substantial alteration in body weight during the exposure. There were no deaths in the groups of rats and mice exposed to 300 ppm DMMP for 2 weeks.

90-Day Inhalation - On the basis of the results obtained during the 2-week continuous inhalation pilot study, exposure concentrations of 250 ppm and 25 ppm were chosen for the 90-day study. Exposure of rats and mice began in late July, 1983. On the third day of exposure to 250 ppm DMMP a number of mice, both male and female, were found dead or in a moribund condition. Gross necropsy and bacteriological tests failed to establish a cause for the deaths. Shortly before the mouse deaths occurred a visible condensate had begun to form on the chamber walls and it was noted that many of the mice that were removed from the study had wet coats. Because of the large number of deaths in the

group of mice exposed to 250 ppm DMMP it was decided to terminate all mice from the study and obtain a new lot for replacement. Due to the sampling requirements at the end of the exposure it was necessary to stagger the introduction of the animals into the chamber. Therefore, at the time of the mouse deaths, the rats had completed approximately 10 days of exposure. At that time the rats exhibited no overt signs of toxicity and their exposure was continued.

TABLE 61. EFFECT OF 2 WEEKS OF EXPOSURE TO 500 PPM DMMP ON RAT ACETYLCHOLINESTERASE ACTIVITY (ΔVbs/min)[ab]

	Plasma	
	Control (N = 5)	500 ppm (N = 5)
Male	0.0323 ± 0.0017	0.0587 ± 0.0017 (182)[c]
Female	0.0989 ± 0.0032	0.0887 ± 0.0032 (90)
	Erythrocyte	
	Control (N = 5)	500 ppm (N = 5)
Male	0.0092 ± 0.0001	0.0079 ± 0.0002 (86)[c]
Female	0.0093 ± 0.0001	0.0068 ± 0.0002 (73)

[a] Acetylcholinesterase activity measured by the method of Ellman et al. (1961).
[b] Mean ± SE, (% of control activity).
[c] N = 4.

TABLE 62. BODY WEIGHTS OF RODENTS EXPOSED TO 300 PPM DMMP CONTINUOUSLY FOR 2 WEEKS[a]

	Exposure Day				
	0	2	6	9	14
Male Rats	228 ± 2	220 ± 2	226 ± 2	228 ± 8	232 ± 8
Female Rats	152 ± 2	152 ± 2	152 ± 2	151 ± 7	151 ± 7
Male Mice	23 ± 2	23 ± 2	25 ± 2	24 ± 2	23 ± 1
Female Mice	20 ± 1	20 ± 2	21 ± 1	21 ± 1	20 ± 1

[a] Mean ± SE, grams (N = 10).

Examination of the exposure records for the study indicated that at the time the mice were dying the relative humidity (RH) in the chamber was unusual (greater than 80%) and the chamber air flow was about 20 CFM. In addition, it was noted that whenever the cloth sleeve on the wet bulb temperature sensor located in the chamber was replaced with a clean sleeve, the RH reading immediately dropped to near 50%, the normal level. The RH value then increased over the next 24 hour period. This information suggested that the condensate in the chamber was probably an azeotropic mixture formed between the DMMP and water vapor. Indeed analysis of the condensate collected from the chamber walls showed a DMMP concentration of about 80%. To reduce the formation of condensate the chamber air flow was increased to 50 CFM. To investigate the cause of the death in the original group of mice additional tests were conducted. Eight C57BL/6 mice were introduced into the 250 ppm DMMP chamber and removed two days later. All eight mice were moribund with wetted fur. Condensate persisted on the chamber walls. The chamber air flow was increased to 80 CFM and eight C57BL/6 male mice and eight B6C3F1 female mice were placed in the chamber to determine if a strain difference existed. Exposure of these mice continued without any deaths for 10 days when two C57BL/6 mice died. On day 11 of exposure three more C57BL/6 mice died and on day 15 of exposure four B6C3F1 mice were found dead. Thus, this response did not appear to be a function of strain difference.

During the period of investigation we observed that the condensate developed whenever the room temperature decreased slightly and deaths most often followed periods of heavy condensation or when vaporization of the condensate occurred during room reheating. The mice housed nearest the windows of the chamber were also the animals that accumulated the most condensate on the fur.

The results indicated that mice could tolerate exposure to 250 ppm DMMP if the formation of condensate was closely controlled. A fourth exposure chamber was then utilized for the exposure of mice, thus permitting additional area to distribute the mice and allow for housing near the center of the chamber away from the walls. Procedures instituted to control the condensation included: increased air flow, close control of room temperature along with redirection of the air conditioning vents, limited use of water in the chambers during cleaning and routine drying of the chamber interior walls and surfaces. These same procedures were also followed in the other chambers and resulted

in the successful completion of the 90-day exposure of mice without significant mortality. The DMMP vapor concentrations achieved for animal exposures are shown in Table 63.

TABLE 63. AVERAGE DMMP CONCENTRATIONS FOR 90-DAY CONTINUOUS EXPOSURES OF ANIMALS

Exposure	Start Date	End Date	Average Conc. (ppm)	SD[a]	Exposure Days
250 ppm-rat	21 Jun 83	20 Sep 83	239	23	92
25 ppm-rat	21 Jun 83	20 Sep 83	24.7	1.8	92
250 ppm-mouse	03 Aug 83	02 Nov 83	247	10	92
25 ppm-mouse	03 Aug 83	02 Nov 83	25.1	1.5	92

[a] Sample standard deviation of daily mean concentrations.

Because of the separate mouse and rat exposure chambers and the restart of the mouse exposures, the concentration averages were calculated based on the respective dates of exposure. Results of routine aerosol analysis indicated a very slight aerosol present through the duration of the exposure.

Male rat body weights are shown in Figure 28. Exposure to 250 ppm DMMP depressed the weight gain of male rats. Upon removal from the exposure, recovery of body weight occurred. Female rats exhibited a similar trend in body weight (Figure 29). Exposure to 250 ppm DMMP resulted in very little weight gain in female rats. Removal from the exposure resulted in substantial weight gain, and at 14 weeks postexposure the body weights of female rats exposed to 250 ppm DMMP were not significantly different from controls. The body weights of male and female rats exposed to 25 ppm DMMP were unaffected by exposure.

Significant ($p < 0.001$) reductions in red blood cell counts, hematocrit and hemoglobin levels were observed in male and female rats exposed to 250 ppm DMMP (Tables 64 and 65, respectively). These parameters also tended to be reduced in the male and female rats exposed to 25 ppm, but the values were not always significantly different from the controls at the 0.05 level of confidence. Clinical parameters indicative of hepatic or renal injury were normal in male rats exposed to either concentration of DMMP. These values were generally reduced in female rats exposed to 250 ppm DMMP when compared to controls. Examination of rat

blood at 3 months postexposure again indicated reduced red blood cell counts, hematocrit, and hemoglobin levels in male rats exposed to DMMP (Table 66). Female rats exposed to DMMP did not display these trends when examined 3 months postexposure (Table 67).

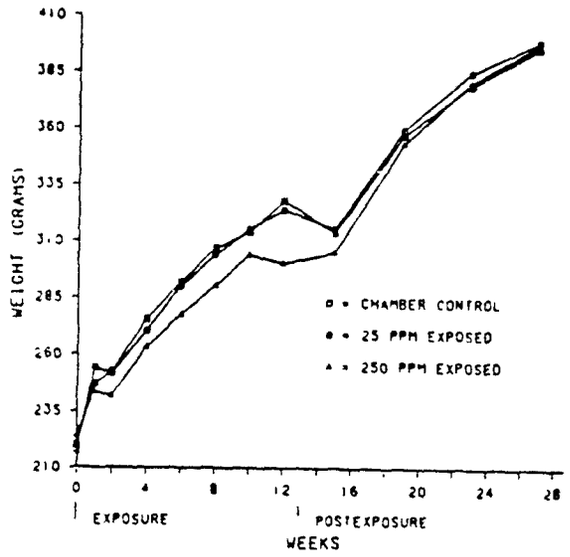


Figure 28. Effect of 90-day continuous exposure to dimethyl methylphosphonate on male rat body weight.

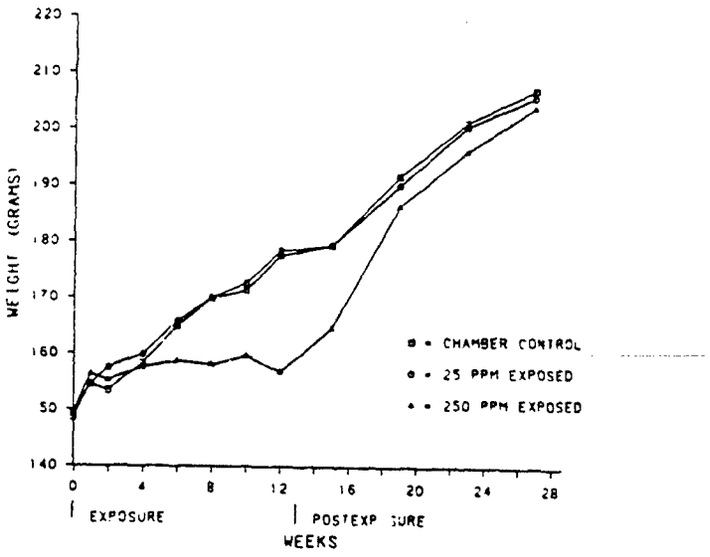


Figure 29. Effect of 90-day continuous exposure to dimethyl methylphosphonate on female rat body weight.

TABLE 64. MALE RAT BLOOD PARAMETERS[a] AFTER 90-DAYS CONTINUOUS EXPOSURE TO DIMETHYL METHYLPHOSPHONATE

	Control (N=15)	25 ppm (N=15)	250 ppm (N=14)
WBC ($x10^3$ cells/mm^3)	7.5 ± 0.3	6.8 ± 0.4	6.5 ± 0.3
RBC ($x10^6$ cells/mm^3)	8.23 ± 0.09	7.91 ± 0.15	7.03 ± 0.11[b]
HGB (g/dl)	15.4 ± 0.1	14.7 ± 0.3	13.8 ± 0.2[b]
HCT (%)	41.0 ± 0.4	39.6 ± 0.8	35.7 ± 0.6[b]
MCV (um^3)	49.9 ± 0.1	50.1 ± 0.3	50.8 ± 0.2[c]
MCH (pg)	18.7 ± 0.1	18.6 ± 0.2	19.6 ± 0.2[c]
MCHC (g/dl)	37.5 ± 0.2	37.1 ± 0.5	38.6 ± 0.5
Glucose (mg/dl)	160 ± 4	156 ± 8	157 ± 8
Tot. Prot. (g/dl)	6.84 ± 0.05	6.52 ± 0.08[d]	6.83 ± 0.09
Albumin (g/dl)	0.98 ± 0.01	1.00 ± 0.02	1.06 ± 0.01[b]
Globulin (g/dl)	5.85 ± 0.05	5.52 ± 0.07[c]	5.76 ± 0.08
BUN (mg/dl)	14.9 ± 0.3	15.9 ± 0.4	13.7 ± 0.4
Creatinine (mg/dl)	0.5 ± 0.02	0.6 ± 0.02	0.6 ± 0.02
Calcium (mg/dl)	10.6 ± 0.1	10.4 ± 0.1	10.8 ± 0.1
SGOT (IU/L)	89 ± 3	88 ± 4	81 ± 2
SGPT (IU/L)	41 ± 2	40 ± 2	40 ± 3
Alk. Phos. (IU/L)	101 ± 2	95 ± 4	95 ± 4
Bilirubin (mg/dl)	0.4 ± 0.01	0.4 ± 0.01	0.4 ± 0.01

[a] Values expressed as mean ± SE.
[b] Statistically different from control at $p < 0.001$.
[c] Statistically different from control at $p < 0.01$.
[d] Statistically different from control at $p < 0.05$.

TABLE 65. FEMALE RAT BLOOD PARAMETERS[a] AFTER 90-DAYS CONTINUOUS EXPOSURE TO DIMETHYL METHYLPHOSPHONATE

	Control (N=15)	25 ppm (N=14)	250 ppm (N=13)
WBC ($x10^3$ cells/mm^3)	5.2 ± 0.3	4.4 ± 0.5	4.1 ± 0.2[b]
RBC ($x10^6$ cells/mm^3)	7.43 ± 0.13	6.91 ± 0.15[b]	6.49 ± 0.09[c]
HGB (g/dl)	14.6 ± 0.2	14.1 ± 0.2	13.4 ± 0.2[c]
HCT (%)	39.8 ± 0.7	37.1 ± 0.9[b]	34.6 ± 0.5[c]
MCV (um^3)	53.5 ± 0.2	53.7 ± 0.2	53.3 ± 0.2
MCH (pg)	19.7 ± 0.3	20.4 ± 0.4	20.7 ± 0.1[b]
MCHC (g/dl)	36.9 ± 0.5	38.0 ± 0.7	38.9 ± 0.3[d]
Glucose (mg/dl)	121 ± 5	118 ± 6	97 ± 5[d]
Tot. Prot. (g/dl)	7.16 ± 0.08	7.10 ± 0.11	7.35 ± 0.06
Albumin (g/dl)	1.09 ± 0.01	1.09 ± 0.02	1.13 ± 0.01
Globulin (g/dl)	6.07 ± 0.07	6.04 ± 0.11	6.22 ± 0.06
BUN (mg/dl)	15.8 ± 0.6	15.0 ± 0.4	12.1 ± 0.7[c]
Creatinine (mg/dl)	0.6 ± 0.01	0.5 ± 0.02[d]	0.4 ± 0.01(13)[c]
Calcium (mg/dl)	10.5 ± 0.1	10.5 ± 0.1	10.1 ± 0.2(11)
SGOT (IU/L)	101 ± 6	101 ± 7	77 ± 2(13)[b]
SGPT (IU/L)	49 ± 4	51 ± 5	37 ± 2(13)[b]
Alk. Phos. (IU/L)	54 ± 2	50 ± 2	37 ± 3(13)[b]
Bilirubin (mg/dl)	0.4 ± 0.01	0.4 ± 0.00	0.4 ± 0.01(13)

[a] Values expressed as mean ± SE.
[b] Statistically different from control at $p < 0.05$.
[c] Statistically different from control at $p < 0.001$.
[d] Statistically different from control at $p < 0.01$.

TABLE 66. MALE RAT BLOOD PARAMETERS[a] 3 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO DIMETHYL METHYLPHOSPHONATE

	Control	25 ppm	250 ppm
WBC ($x10^3$ cells/mm^3)	7.2 ± 0.3	6.5 ± 0.3	6.4 ± 0.2
RBC ($x10^6$ cells/mm^3)	8.33 ± 0.10	7.83 ± 0.09[b]	7.41 ± 0.10[c]
HGB (g/dl)	16.0 ± 0.2	15.0 ± 0.1[c]	14.9 ± 0.1c
HCT (%)	41.0 ± 0.5	38.1 ± 0.5[c]	36.1 ± 0.5[c]
MCV (um^3)	49.2 ± 0.2	48.8 ± 0.2	48.8 ± 0.7
MCH (pg)	19.2 ± 0.1	19.2 ± 0.2	20.0 ± 0.1[b]
MCHC (g/dl)	39.1 ± 0.2	39.4 ± 0.6	41.1 ± 0.2[c]
Glucose (mg/dl)	152 ± 10	172 ± 7	161 ± 9
Tot. Prot. (g/dl)	7.62 ± 0.13	7.67 ± 0.06	7.73 ± 0.07
Albumin (g/dl)	1.08 ± 0.01	1.10 ± 0.01	1.08 ± 0.02
Globulin (g/dl)	6.55 ± 0.12	6.57 ± 0.05	6.65 ± 0.06
BUN (mg/dl)	18.9 ± 0.9	17.8 ± 0.7	16.2 ± 0.8
Creatinine (mg/dl)	0.6 ± 0.02	0.5 ± 0.04	0.5 ± 0.03
Calcium (mg/dl)	10.8 ± 0.1	11.0 ± 0.1	10.8 ± 0.1
SGOT (IU/L)	115 ± 12	96 ± 8	83 ± 5[d]
SGPT (IU/L)	95 ± 14	66 ± 4	57 ± 4[d]
Alk. Phos. (IU/L)	104 ± 7	103 ± 2	90 ± 4
Bilirubin (mg/dl)	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02

[a] Values expressed as mean ± SE.
[b] Statistically different from control at $p < 0.01$.
[c] Statistically different from control at $p < 0.001$.
[d] Statistically different from control at $p < 0.05$.

TABLE 67. FEMALE RAT BLOOD PARAMETERS[a] 3 MONTHS AFTER 90-DAY CONTINUOUS EXPOSURE TO DIMETHYL METHYLPHOSPHONATE

	Control	25 ppm	250 ppm
WBC ($x10^3$ cells/mm^3)	5.5 ± 0.3	5.1 ± 0.1	5.2 ± 0.3
RBC ($x10^6$ cells/mm^3)	7.04 ± 0.09	6.91 ± 0.09	6.90 ± 0.08
HGB (g/dl)	15.2 ± 0.2	15.0 ± 0.2	15.0 ± 0.2
HCT (%)	38.4 ± 0.5	37.9 ± 0.5	38.3 ± 0.5
MCV (um^3)	54.5 ± 0.2	54.9 ± 0.1	55.6 ± 0.2[b]
MCH (pg)	21.5 ± 0.2	21.7 ± 0.1	21.7 ± 0.1
MCHC (g/dl)	39.6 ± 0.3	39.6 ± 0.2	39.1 ± 0.1
Glucose (mg/dl)	164 ± 2	127 ± 5[b]	143 ± 6[c]
Tot. Prot. (g/dl)	7.40 ± 0.18	7.44 ± 0.07	7.41 ± 0.06
Albumin (g/dl)	1.08 ± 0.02	1.09 ± 0.01	1.09 ± 0.01
Globulin (g/dl)	6.32 ± 0.16	6.35 ± 0.06	6.33 ± 0.06
BUN (mg/dl)	16.8 ± 0.7	16.0 ± 1.0	14.9 ± 0.6
Creatinine (mg/dl)	0.5 ± 0.03	0.4 ± 0.02[c]	0.5 ± 0.02
Calcium (mg/dl)	10.5 ± 0.1	10.5 ± 0.1	10.4 ± 0.1
SGOT (IU/L)	89 ± 3	80 ± 2	87 ± 7
SGPT (IU/L)	50 ± 2	45 ± 3	49 ± 4
Alk. Phos. (IU/L)	125 ± 19	63 ± 3[c]	78 ± 5
Bilirubin (mg/dl)	0.1 ± 0.02	0.2 ± 0.05	0.1 ± 0.02

[a] Values expressed as mean ± SE.
[b] Statistically different from control at $p < 0.001$.
[c] Statistically different from control at $p < 0.05$.

Organ weights obtained from rats at exposure termination are shown in Table 68. Exposure to 250 ppm DMMP significantly increased the liver and kidney weights in both male and female rats. Increased kidney weight was also noted in the male rats exposed to 25 ppm. This effect was not seen in female rats exposed at the 25 ppm level. Testicular atrophy was evident in the male rats exposed to the higher concentration, while the lower exposure concentration had no effect on testes weight. Body weights of male and female rats exposed to 250 ppm were significantly lower than controls at exposure termination. Organ weights measured at 3 months postexposure are shown in Table 69. Increased liver and kidney weights and decreased testes weight persisted in male rats exposed to 250 ppm DMMP. Interestingly, the testes weight of the control male rats at 3 months postexposure had shown a slight increase compared to the weight at exposure termination, while the testes weight of the male rats exposed to 250 ppm DMMP decreased over the same time period. DMMP exposed female rat organ weights measured at 3 months postexposure were not significantly different from controls.

TABLE 68. RAT ORGAN WEIGHTS[a] MEASURED AFTER 90-DAY CONTINUOUS EXPOSURE TO DIMETHYL METHYLPHOSPHONATE

	Male		
	Control (N=12)	25 ppm (N=12)	250 ppm (N=12)
Fasted Body wt (g)	319 ± 3	313 ± 4	290 ± 7[b]
Liver wt (g)	8.23 ± 0.13	8.25 ± 0.18	10.41 ± 0.26[b]
Liver/100 g body wt	2.58 ± 0.03	2.64 ± 0.05	3.61 ± 0.12[b]
Testes wt (g)	3.10 ± 0.03	3.10 ± 0.03	2.54 ± 0.13[b]
Testes/100 g body wt	0.97 ± 0.01	0.99 ± 0.01	0.87 ± 0.04[c]
Kidney wt (g)	2.13 ± 0.03	2.36 ± 0.06[d]	2.91 ± 0.04[b]
Kidney/100 g body wt	0.67 ± 0.01	0.76 ± 0.02[c]	1.01 ± 0.03[b]
Spleen wt (g)	0.67 ± 0.03	0.62 ± 0.01	0.65 ± 0.02
Spleen/100 g body wt	0.21 ± 0.01	0.20 ± 0.004	0.23 ± 0.01
	Female		
	Control (N=15)	25 ppm (N=15)	250 ppm (N=14)
Fasted Body wt (g)	175 ± 2	175 ± 3	146 ± 3[b]
Liver wt (g)	4.32 ± 0.07	4.32 ± 0.08	4.73 ± 0.10[d]
Liver/100 g body wt	2.47 ± 0.04	2.47 ± 0.04	3.25 ± 0.06[b]
Kidney wt (g)	1.21 ± 0.02	1.25 ± 0.02	1.47 ± 0.03[b]
Kidney/100 g body wt	0.69 ± 0.01	0.72 ± 0.02	1.01 ± 0.02[b]
Spleen wt (g)	0.40 ± 0.01	0.42 ± 0.01	0.34 ± 0.01[b]
Spleen/100 g body wt	0.23 ± 0.004	0.24 ± 0.003	0.23 ± 0.004

[a] Values expressed as mean ± SE.
[b] Statistically different from control value at $p < 0.001$.
[c] Statistically different from control value at $p < 0.05$.
[d] Statistically different from control value at $p < 0.01$.

TABLE 69. RAT ORGAN WEIGHTS[a] 3 MONTHS AFTER 90-DAYS CONTINUOUS EXPOSURE TO DIMETHYL METHYLPHOSPHONATE

	Male		
	Control (N=10)	25 ppm (N=10)	250 ppm
Fasted Body wt (g)	373 ± 6	377 ± 5	382 ± 4
Liver wt (g)	9.03 ± 0.35	9.40 ± 0.23	10.29 ± 0.34[b]
Liver/100 g body wt	2.42 ± 0.07	2.49 ± 0.05	2.69 ± 0.07[b]
Testes wt (g)	3.24 ± 0.03	3.21 ± 0.05	2.14 ± 0.16[c]
Testes/100 g body wt	0.87 ± 0.02	0.85 ± 0.01	0.56 ± 0.04[c]
Kidney wt (g)	2.43 ± 0.04	2.39 ± 0.05	2.68 ± 0.07[d]
Kidney/100 g body wt	0.65 ± 0.01	0.64 ± 0.01	0.70 ± 0.01[b]
Spleen wt (g)	0.64 ± 0.01	0.67 ± 0.01	0.71 ± 0.01[d]
Spleen/100 g body wt	0.17 ± 0.003	0.18 ± 0.003	0.19 ± 0.003[d]
	Female		
	Control (N=10)	25 ppm (N=10)	250 ppm
Fasted Body wt (g)	191 ± 2	194 ± 2	195 ± 3
Liver wt (g)	5.30 ± 0.20	4.81 ± 0.08	5.14 ± 0.11
Liver/100 g body wt	2.78 ± 0.12	2.48 ± 0.04	2.64 ± 0.05
Kidney wt (g)	1.41 ± 0.03	1.41 ± 0.03	1.46 ± 0.03
Kidney/100 g body wt	0.74 ± 0.01	0.72 ± 0.02	0.75 ± 0.02
Spleen wt (g)	0.43 ± 0.01	0.47 ± 0.01[b]	0.45 ± 0.01
Spleen/100 g body wt	0.23 ± 0.003	0.24 ± 0.003[b]	0.23 ± 0.01

[a] Values expressed as mean ± SE.
[b] Statistically different from control value at $p < 0.05$.
[c] Statistically different from control value at $p < 0.001$.
[d] Statistically different from control value at $p < 0.01$.

Although the tissues collected from the rats and mice at exposure termination and 3 months postexposure have not yet been examined, the results obtained to date suggest that a level of 250 ppm DMMP is unsafe for prolonged inhalation exposure. This

is indicated by the reduced weight gain of male and female rats during exposure as well as altered organ weights, particularly decreased testes weight in male rats. Information from the ongoing observations during the postexposure period will be presented in future annual reports.

EVALUATION OF THE 21-DAY REPEATED DOSE DERMAL TOXICITY OF HOUGHTO-SAFE 273 AND DURAD MP280

The Navy Medical Research Institute/Toxicology Detachment (NMRI/TD) requested that the THRU conduct a series of toxicity studies with three hydraulic fluids. Two of the fluids, Fyrquel 220 and Durad MP280, are phosphate ester based while Houghto-Safe 273 is water-glycol based. As part of the toxicity evaluation of the hydraulic fluids, repeated dose dermal exposures were conducted. The results of the 21-day test with Fyrquel 220 were reported in a previous annual report (MacEwen and Vernot, 1983). This report presents the results obtained with the other two hydraulic fluids, Houghto-Safe 273 and Durad MP280.

The basic experimental design for the repeated dermal tests was similar for all of the hydraulic fluids tested. Because of differences in solubility characteristics and toxicity, the vehicles and concentrations chosen varied among the hydraulic fluids. It was not possible to conduct simultaneous testing of both sexes because of limited space for housing. For either hydraulic fluid the male rabbits were the first tested, followed by the female rabbits. This sometimes resulted in lowering in the concentrations used for females because of high mortality in males. Also, some additional blood tests were conducted on female rabbits based on the results obtained with the male rabbits. Experimental groups consisted of 10 male and 10 female New Zealand White rabbits weighing between 2-3 kg at the start of the study. Each rabbit received an occluded patch on weekdays for three consecutive weeks for a total of 15 applications over a 21 day period. Occlusion lasted for 6 hours daily. Hair on the back was clipped from the animal as necessary with application of the material over the clipped area. A single 4 x 4 gauze patch was placed over the application area and the entire trunk of the animal was wrapped with an impervious polyethylene material held in place with surgical tape. Upon removal of the wrapping, the skin was wiped in order to remove excess test material. The rabbits were restrained in stocks during dosing to prevent disturbance of the wrap.

The exposed skin area of five animals from each group was abraded once each week throughout the study. The abrasions did not penetrate the stratum corneum. Dermal irritation scores according to the method of Draize (1959) were recorded daily immediately prior to the next application of the test material.

Body weights and food consumption were measured and recorded daily prior to dosing. Toxic signs were also recorded when observed. Hematology and serum chemistry determinations were conducted on blood obtained via cardiac puncture at the beginning (shortly before the first application) and at the end of the 21-day study period. When possible, blood was also collected from moribund animals.

At necropsy, liver, kidney, heart, and spleen weights were recorded and these tissues were taken for microscopic examination along with samples of skin, ovaries, testes, thyroid, adrenals, brain, and other lesions when observed.

Results

Houghto-Safe 273

Male rabbits were exposed to Houghto-Safe 273 at concentrations of 5 ml/kg, 2.5 ml/kg, or 0.5 ml/kg. Female rabbits were exposed to Houghto-Safe 273 at concentrations of 4.0 ml/kg, 2.0 ml/kg, or 0.5 ml/kg. Water was used as the vehicle for the lower two exposure concentrations for each sex and was also applied to the rabbits serving as controls. The upper level concentration was applied neat. Dose volumes were kept constant for all groups within a sex. For example, the high level male rabbit group received 5 ml/kg neat, the 2.5 ml/kg group received 5 ml/kg of a 50% solution, and the 0.5 ml/kg group received 5.0 ml/kg of a 10% solution. Females received similar treatment except the dose volumes were based on 4.0 ml/kg.

The original group size was 10 animals/group, but during the study we found that a small number of the rabbits had been mis-sexed by the supplier. Three female rabbits were included in the male rabbit tests and one male rabbit was used in the female rabbit tests. All data from these animals were excluded from analysis. Therefore, the group sizes were adjusted accordingly. Male rabbits: 9 controls and 10 in each of the exposed groups; female rabbits: 10 controls and 9 in each of the exposed groups.

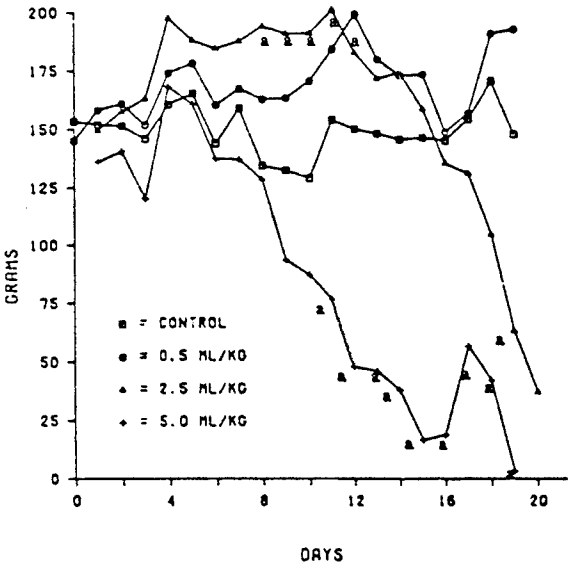
One control male rabbit died after 9 treatments, while one male rabbit in the 2.5 ml/kg died after 3 doses. There were no deaths in the 0.5 ml/kg treatment group. Both rabbits that died exhibited mild diarrhea and loss of appetite and their deaths appeared to be unrelated to treatment. Gross necropsy and bacteriology results failed to suggest a cause of death. Significant mortality occurred in the highest level exposure group (5 ml/kg) where four male rabbits died during the course of the study (1 after 10 doses, 1 after 13 doses, 2 after 14 doses). Symptomatology of these animals included diarrhea, loss of appetite, lethargy, jaundice, and bright yellow urine. Because of the general depressed condition of the rabbits remaining in the 5 ml/kg exposure group, they were killed one day earlier than originally scheduled to insure collection of fresh blood and tissue samples. One female rabbit dosed at 4.0 ml/kg died after 15 applications, just one day prior to the scheduled necropsy. No deaths occurred in any of the other female groups. No significant skin irritation was noted during the studies.

As shown in Figure 30, a marked reduction in food consumption was seen in the group of male rabbits dosed at 5.0 ml/kg. After having a period of increased food consumption compared to controls, a similar pattern of reduced food consumption was seen at the 2.5 ml/kg exposure level. This decline occurred later in the study than that seen in the 5.0 ml/kg exposure group. Female rabbits demonstrated a similar trend in food consumption (Figure 31). Females dosed at 4 ml/kg showed decreased food consumption approximately mid-way through exposure. The females dosed at 2.0 ml/kg demonstrated a reduced food consumption pattern during the latter phase of exposure. Neither of the male or female groups exposed to 0.5 ml/kg exhibited any alteration in food consumption.

Concomitant with the reduced food consumption were substantially decreased body weights in male rabbits dosed at 5.0 ml/kg (Figure 32) and females dosed at 4.0 ml/kg (Figure 33). Male and female rabbits dosed at 2.5 ml/kg and 2.0 ml/kg, respectively, exhibited mild weight loss during the last portion of the exposure period. However, neither group was significantly different from its respective controls at termination. Exposure to 0.5 ml/kg did not affect the weight gain of male or female rabbits.

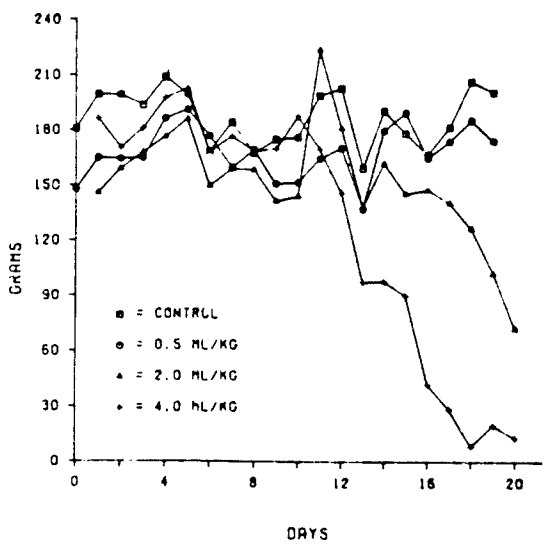
Analysis of blood data collected at necropsy indicated probable liver injury in male rabbits dosed at concentrations of 2.5 ml/kg or greater (Table 70). Elevated SGOT, SGPT, and total bilirubin levels were evident in male rabbits treated at 5.0

ml/kg. These parameters were also increased at the 2.5 ml/kg exposure level, but the values were not found to be significantly different from controls.



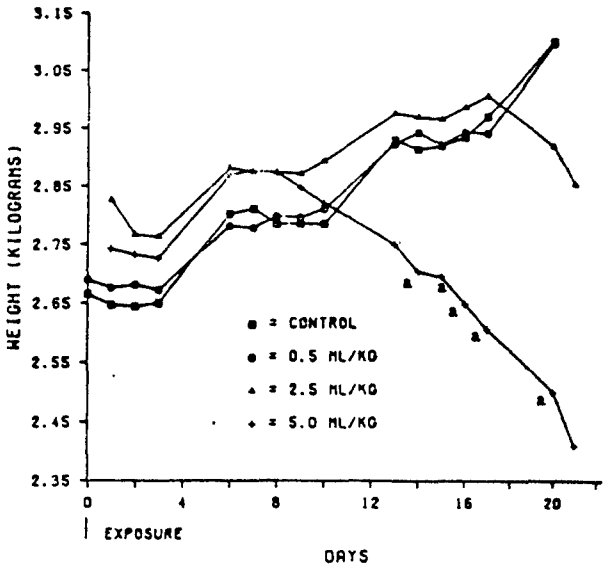
[a] Statistically different from control value at $p < 0.05$.

Figure 30. Effect of Houghto-Safe 273 dermal exposure on male rabbit food consumption.



[a] Statistically different from control value $p < 0.05$.

Figure 31. Effect of Houghto-Safe 273 dermal exposure on female rabbit food consumption.



[a]Statistically different from control value at $p < 0.05$.

Figure 32. Effect of Houghto-Safe 273 dermal exposure on male rabbit body weight.

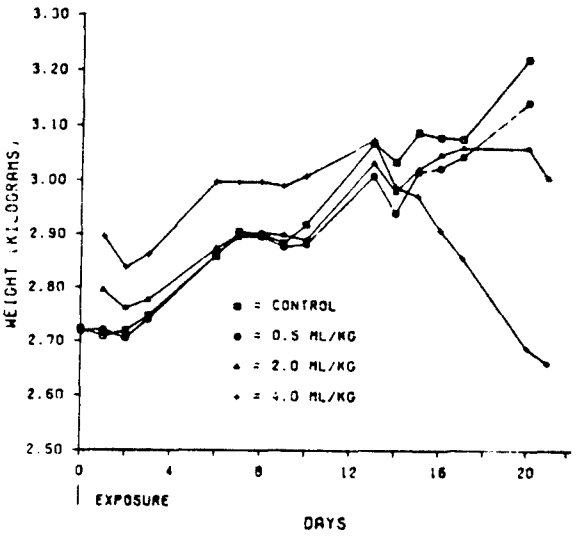


Figure 33. Effect of Houghto-Safe 273 dermal exposure on female rabbit body weight.

One male rabbit in the 2.5 ml/kg treatment group had an SGOT value of over 1400 IU/L, an SGPT level of 260 IU/L, and total bilirubin concentration of 4.6 mg/dl. Five of the six male rabbits from the 5.0 ml/kg group alive at study termination had SGOT levels over 250 IU/L and total bilirubin concentrations over 1.5 mg/dl. Additional evidence of liver injury was seen in the significant reductions in total protein values of the upper level dose groups. Five of the 9 serum samples collected from the 2.5 ml/kg group and 3 of 8 samples from the 5.0 ml/kg group were characterized as lipemic. Although not statistically significant at the 0.05 level of confidence, BUN and creatinine levels of the 5.0 ml/kg group were higher than controls. White blood cell counts of the male rabbits dosed at 5.0 ml/kg were significantly greater than the controls (Table 70). Differential counts indicated a mild increase in neutrophils. The red blood cell count, hematocrit, and hemoglobin values of the 2.5 ml/kg exposure group were elevated compared to controls. However, no dose response was indicated as values of the 5.0 ml/kg exposure group was similar to controls at the 0.05 level of significance.

TABLE 70. EFFECT OF REPEATED DERMAL EXPOSURE TO HOUGHTO-SAFE 273 ON MALE RABBIT BLOOD PARAMETERS[a]

	Control	0.05 ml/kg	2.5 ml/kg	5.0 ml/kg
RBC ($x10^6$ cells/mm^3)	5.67 ± 0.31	6.13 ± 0.15	7.04 ± 0.38[b]	6.33 ± 0.29
WBC ($x10^3$ cells/mm^3)	8.5 ± 0.6	9.7 ± 0.8	8.6 ± 1.0	14.5 ± 2.3[c]
HCT(%)	37.4 ± 2.9	39.4 ± 0.8	46.0 ± 1.6[b]	40.6 ± 1.7
HGB(g/dl)	13.2 ± 0.8	13.8 ± 0.3	16.3 ± 0.5[b]	14.2 ± 0.7
MCV(μm^3)	65.8 ± 0.6	64.3 ± 1.0	65.8 ± 1.6	64.2 ± 0.8
MCH(pg)	23.3 ± 0.2	22.5 ± 0.4	23.3 ± 0.7	22.5 ± 0.2[c]
MCHC(g/dl)	35.3 ± 0.2	34.9 ± 0.1	35.4 ± 0.4	35.1 ± 0.3
Glucose(mg/dl)	125 ± 4	137 ± 4	114 ± 10	107 ± 19
BUN(mg/dl)	18.9 ± 0.7	16.0 ± 1.4	15.2 ± 1.4	31.3 ± 8.2
Creatin.(mg/dl)	1.2 ± 0.1	1.5 ± 0.3	1.5 ± 0.1	2.3 ± 0.5
Tot. Prot.(g/dl)	5.85 ± 0.14	5.86 ± 0.22	4.76 ± 0.40	3.69 ± 0.20[b]
SGOT(IU/L)	54 ± 10	67 ± 15	302 ± 173	401 ± 83[b]
SGPT(IU/L)	44 ± 4	41 ± 6	97 ± 26	131 ± 49
Bilirub.(mg/dl)	0.4 ± 0.01	0.5 ± 0.02	1.6 ± 0.52	3.3 ± 0.9

[a] Values expressed as mean ± SE, N=6 to 9.
[b] Statistically different from control at $p < 0.01$.
[c] Statistically different from control at $p < 0.05$.

The effect of Houghto-Safe 273 exposure on female rabbit blood is shown in Table 71. Increased red blood cell counts and hemoglobin were found in female rabbits dosed at 2.0 ml/kg or greater. Two of the female rabbits sampled from the 4.0 ml/kg dose group had white blood cell counts in excess of 15 x 10^3 cells/mm^3. While these values contributed to a moderate elevation in the mean WBC count for the group, the mean was not found to be significantly greater than the control value. Hepatotoxicity in female rabbits exposed to Houghto-Safe 273 was indicated by a number of blood clinical chemistry values. Alkaline phosphatase and SGOT were both markedly increased in female rabbits dosed at 4.0 ml/kg. This trend was also apparent in rabbits dosed at 2.0 ml/kg although the value for SGOT was not statistically different from the control. Elevations in the mean SGPT and bilirubin levels for the 4.0 ml/kg exposure group were primarily due to single values which exceeded the range of other animals in the group.

TABLE 71. EFFECT OF REPEATED DERMAL EXPOSURE TO HOUGHTO-SAFE 273 ON FEMALE RABBIT BLOOD PARAMETERS[a]

	Control	0.05 ml/kg	2.0 ml/kg	4.0 ml/kg
RBC (x10^6 cells/mm^3)	5.80 ± 0.06	4.98 ± 0.11	6.51 ± 0.33	6.47 ± 0.19[b]
WBC (x10^3 cells/mm^3)	8.6 ± 0.3	9.1 ± 0.4	7.2 ± 0.8	10.2 ± 1.2
HCT (%)	38.8 ± 0.6	39.2 ± 0.6	42.1 ± 1.8	42.7 ± 1.2
HGB (g/dl)	13.3 ± 0.2	13.4 ± 0.2	14.9 ± 0.5[c]	15.1 ± 0.5[c]
MCV (μm^3)	66.9 ± 0.4	65.6 ± 0.6	64.8 ± 1.0	65.3 ± 1.0
MCH (pg)	23.0 ± 0.1	22.5 ± 0.2	23.1 ± 0.4	23.3 ± 0.3
MCHC (g/dl)	34.3 ± 0.2	34.3 ± 0.1	35.6 ± 0.5	35.6 ± 0.4
Glucose(mg/dl)	134 ± 6	132 ± 3	116 ± 4	121 ± 2[c]
BUN (mg/dl)	20.3 ± 1.0	19.7 ± 1.4	17.7 ± 1.3	18.4 ± 1.4
Creatin.(mg/dl)	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.4 ± 0.1
Tot.Prot.(g/dl)	5.88 ± 0.19	5.75 ± 0.16	5.47 ± 0.29	5.14 ± 0.20[c]
SGOT (IU/L)	56 ± 10	39 ± 3	117 ± 31	136 ± 25[c]
SGPT (IU/L)	53 ± 10	39 ± 5	67 ± 10	101 ± 39
Alk.Phos.(IU/L)	204 ± 16	190 ± 24	391 ± 42[b]	421 ± 61[b]
Bilirub.(mg/dl)	0.4 ± 0.01	0.4 ± 0.01	0.5 ± 0.04	0.8 ± 0.3
Cholest.(mg/dl)	127 ± 21	103 ± 12	115 ± 17	325 ± 45[b]

[a] Values expressed as mean ± SE, N=4 to 10.
[b] Statistically different from control at $p < 0.01$.
[c] Statistically different from control at $p < 0.05$.

Due to the apparent liver toxicity seen in the male rabbit studies completed prior to the female rabbit tests, serum cholesterol measurements were added to the list of clinical chemistry tests conducted by NMRI/TD. Female rabbits receiving 4.0 ml/kg Houghto-Safe 273 had substantially increased cholesterol levels when compared to controls. No effect was noted at the lower exposure levels. Reduced total protein levels in the rabbits dosed at 4.0 ml/kg may either indicate liver injury or the depressed nutritional state of the animals. BUN and creatinine levels of exposed female rabbits were comparable to controls.

Organ weights obtained from rabbits at the end of the study are shown in Table 72. Although there were some statistically significant changes in the exposed male and female rabbits when compared to controls, there did not appear to be a consistent trend in organ weight changes for either sex. For example, decreased heart weight was noted in male rabbits treated at concentrations of 2.5 ml/kg or greater, while no heart weight effect was seen in female rabbits. Liver weights were reduced in female rabbits exposed to the two higher levels of Houghto-Safe 273, but male rabbits did not exhibit this trend. Significantly increased kidney weight was seen only in the male rabbits treated with 5.0 ml/kg Houghto-Safe 273. The only consistent effect noted was in the body weights of male and female rabbits from the 5.0 ml/kg and 4.0 ml/kg exposure groups, respectively, which were significantly less than controls.

Gross examination at the time of necropsy revealed firm livers in male rabbits receiving doses of 2.5 ml/kg or greater. Many of the livers from male rabbits dosed at 5.0 ml/kg had a yellow cast and accentuated lobular architecture.

The results of the repeated dermal exposure to Houghto-Safe 273 show significant toxicity at doses of 2.0 ml/kg or greater. Indications included reduced food consumption with concomitant weight loss. Significant elevations in blood chemistry parameters of liver function suggest biliary obstruction or hepatocellular damage. There was also some suggestion of kidney damage in male rabbits dosed at 5.0 ml/kg. The 0.5 ml/kg exposure level appeared to have no effect on the rabbits. Tissues taken for microscopic examination are presently being processed. Results of these examinations will help clarify the extent and nature of the apparent liver and kidney injury resulting from repeated dermal contact with Houghto-Safe 273.

TABLE 72. EFFECT OF REPEATED DERMAL EXPOSURE TO HOUGHTO-SAFE 273 ON RABBIT ORGAN WEIGHT[a]

	Male			
	Control (N = 9)	0.5 ml/kg (N = 9)	2.5 ml/kg (N = 8)	5.0 ml/kg (N = 5)
Fasted Body wt (kg)	3.00 ± 0.59	3.00 ± 0.55	2.82 ± 0.92	2.41 ± 1.23[b]
Heart wt (g)	9.27 ± 0.83	9.83 ± 1.18	6.70 ± 0.40[c]	5.97 ± 0.35[b]
Heart/100 g body wt.	0.31 ± 0.03	0.33 ± 0.05	0.24 ± 0.01[c]	0.25 ± 0.02
Liver wt (g)	93.60 ± 6.24	98.70 ± 6.84	94.00 ± 4.22	81.60 ± 5.10
Liver/100 g body wt.	3.11 ± 0.17	3.32 ± 0.25	3.34 ± 0.12	3.40 ± 0.19
Spleen wt (g)	1.14 ± 0.10	1.27 ± 0.07	1.07 ± 0.09	0.98 ± 0.11
Spleen/100 g body wt.	0.04 ± 0.003	0.04 ± 0.002	0.04 ± 0.003	0.04 ± 0.004
Kidney wt (g)	17.87 ± 0.83	18.47 ± 1.17	17.67 ± 1.06	18.10 ± 2.05
Kidney/100 g body wt.	0.60 ± 0.02	0.62 ± 0.04	0.63 ± 0.04	0.74 ± 0.05[b]

	Female			
	Control (N = 9)	0.5 ml/kg (N = 10)	2.0 ml/kg (N = 10)	4.0 ml/kg (N = 9)
Fasted Body wt (kg)	3.14 ± 0.75	3.07 ± 0.05	2.94 ± 0.12	2.64 ± 0.08[b]
Heart wt (g)	7.08 ± 0.39	7.25 ± 0.42	7.25 ± 0.42	6.25 ± 0.35
Heart/100 g body wt.	0.23 ± 0.01	0.24 ± 0.01	0.25 ± 0.02	0.24 ± 0.01
Liver wt (g)	110.05 ± 9.77	98.68 ± 4.93	86.74 ± 4.92[c]	75.50 ± 3.45[b]
Liver/100 g body wt.	3.48 ± 0.25	3.21 ± 0.11	2.94 ± 0.10	2.87 ± 0.12[c]
Spleen wt (g)	1.28 ± 0.17	1.44 ± 0.11	1.22 ± 0.07	1.15 ± 0.11
Spleen/100 g body wt.	0.04 ± 0.01	0.05 ± 0.003	0.04 ± 0.002	0.04 ± 0.004
Kidney wt (g)	17.99 ± 1.25	16.69 ± 0.81	16.37 ± 0.72	17.16 ± 0.88
Kidney/100 g body wt.	0.57 ± 0.03	0.54 ± 0.02	0.56 ± 0.03	0.66 ± 0.04

[a] Expressed as mean ± SE.
[b] Statistically different from control at $p < 0.01$.
[c] Statistically different from control at $p < 0.05$.

Durad MP280

Prior to initiation of the Durad MP280 21-day study, a range-finding test was conducted with a small number of rabbits to establish probable effect and no-effect levels. For this range-finding work a total of eight occluded doses were applied to rabbits over a two week period. The results of these tests are shown in Table 73. Based on the severity of weight loss and symptomatology a dose of 1.0 ml/kg was unsuitable for a 21-day study. The next lower dose (0.75 ml/kg) depressed weight gain, produced moderate cholinesterase depression and mild toxic symptoms. These effects were considered acceptable for the required upper "effect level". The 0.1 ml/kg dose did not alter weight gain and failed to produce visible signs of toxicity. Although cholinesterase activity at the 0.1 ml/kg level was not measured, this level appeared to be an acceptable "no effect" level. The range-finding study also indicated that neat isopropanol was an unsuitable vehicle for repeated occluded patch application, causing skin irritation and exfoliation. Based on the results of the range-finding study, concentrations of 0.1 ml/kg, 0.4 ml/kg, and 0.8 ml/kg were selected for the 21-day dermal toxicity test with male rabbits.

For comparative purposes it would have been highly desirable to use the same vehicle for the Durad MP280 as that used for the Fyrquel 220 tests, since both are phosphate ester based materials. However, the severity of the skin irritation produced by the corn oil vehicle used in the Fyrquel 220 study precluded this possibility. Mineral oil was unsuitable as an alternative non-toxic vehicle because Durad MP280 was immiscible in straight mineral oil, producing an emulsion that inhibited uniform dosing. We found that a solution of 50% isopropanol and 50% mineral oil produced a homogeneous vehicle that allowed for uniform dosing. A 2 ml/kg dose volume was used. This was considered to be the minimum amount necessary to satisfy the EPA guideline that an area equivalent to 10% of the body surface should be treated. All Durad MP280 concentrations were applied as dilutions in the isopropanol/mineral oil vehicle. Because of reduced Durad MP280 concentrations used in the female rabbit tests, it was possible to reduce the isopropanol concentration. The vehicle for the female rabbit tests was 20% isopropanol and 80% mineral oil, with Durad MP280 concentrations of 0.04 ml/kg, 0.1 ml/kg, and 0.4 ml/kg.

TABLE 73. RANGE-FINDING DURAD MP28C REPEATED DERMAL EXPOSURE IN RABBITS

Dose (ml/kg)[a]	Weight (kg)[b]		Cholinesterase[c]		Symptoms
	Init.	Final	Erythrocyte	Plasma	
0	2.2	2.5	0.0102	0.0498	Moderate to severe erythema & exfoliation
0.1	2.0	2.3	--	--	Mild erythema & exfoliation
0.25	1.9	2.4	0.0035	0.0298	Mild erythema & exfoliation
0.50	1.7	2.0	--	--	Mild erythema & exfoliation
0.75	1.8	1.8	0.0013	0.0165	Mild tremors (1 rabbit)
1.0	2.1	1.8	--	--	Diarrhea & tremors (all rabbits)

[a] Dosed in 2 ml/kg volume with isopropanol as the vehicle.
[b] Mean, N = 3.
[c] Mean, N = 3; N = 2 at the 0.25 ml/kg dose. Units are ABS/min.

Two rabbits, one from the 0.1 ml/kg female exposure group and one from the 0.4 ml/kg female exposure group, were determined to be male rabbits. Data from these animals were excluded from analysis. All other groups of male and female rabbits contained 10 animals.

All 10 of the male rabbits treated at the 0.8 ml/kg level died or were sacrificed in a moribund condition prior to completion of the 21-day study. Signs of toxicity in these animals included loss of appetite, lethargy, and muscle weakness. The two rabbits from the 0.4 ml/kg dose group that died failed to exhibit any symptoms of toxicity other than slightly reduced food

consumption prior to death. Three rabbits were killed due to injuries incurred while the rabbits were in the stocks. With reduced dose levels no mortality occurred in the female rabbits.

The majority of the male rabbits in all groups, including controls, developed mild to moderate epidermal exfoliation during the second week of the study. This condition subsided during the third week of dosing. Female rabbits dosed with the vehicle containing a lower isopropanol concentration developed signs of irritation far less severe than the male rabbits. The skin irritation was undoubtedly related to the isopropanol present in the vehicle and was not considered substantial enough to reduce dosage or terminate application.

The body weight curves obtained for male rabbits are shown in Figure 34. Severe weight loss was evident in the male rabbits dosed at 0.8 ml/kg. Weight gain of the 0.4 ml/kg treatment group was depressed during the latter part of the dosing period, although the weights of the male rabbits in the 0.4 ml/kg group were not statistically different from controls at the 0.05 level. Female rabbits dosed at 0.4 ml/kg also demonstrated reduced weight gain during the latter part of the dosing period when compared to controls (Figure 35). The body weight gains of male and female rabbits dosed at concentrations of 0.1 ml/kg or less were unaffected by exposure.

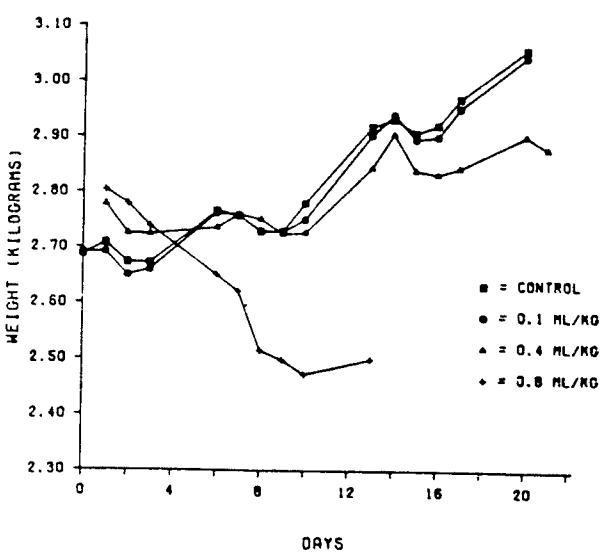


Figure 34. Effect of repeated dermal exposure to Durad MP280 on male rabbit body weight.

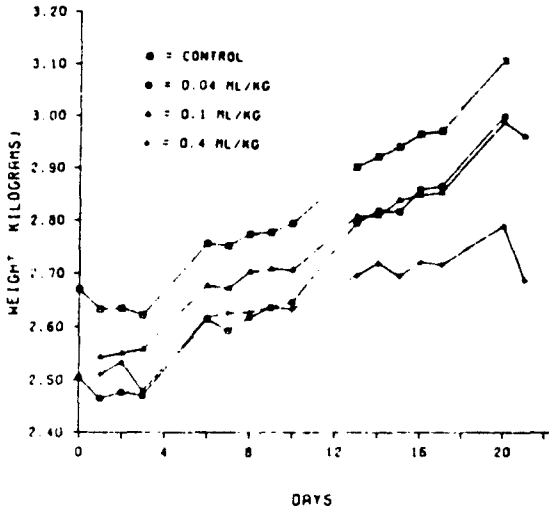


Figure 35. Effect of repeated dermal exposure to Durad MP280 on female rabbit body weight.

Food consumption curves are shown in Figures 36 and 37 for male and female rabbits, respectively. A significant reduction in food consumption was noted in male rabbits dosed at 0.8 ml/kg. Male rabbits dosed at 0.4 ml/kg exhibited reduced food consumption during the last week of dosing; however, female rabbits at the 0.4 ml/kg did not show food consumption reductions. Rabbits dosed with 0.1 ml/kg or less did not show altered food consumption.

The effect of Durad MP280 exposure on male rabbit blood is shown in Table 74. Decreased total protein, albumin, globulin, and alkaline phosphatase were seen at the 0.8 ml/kg treatment level. Although not statistically different at $p < 0.05$, these values were also decreased in the 0.4 ml/kg level when compared to controls. Significant increases in BUN and creatinine levels were seen in female rabbits at the 0.4 ml/kg treatment level (Table 75). Female rabbits dosed at 0.04 ml/kg and 0.1 ml/kg had BUN and creatinine levels equal to controls. Total protein and globulin levels were increased in the two higher female rabbit exposure groups, but the increases were not dose related. Creatinine phosphokinase (CPK) was measured in female rabbits using blood drawn from ear veins. Increased activity of this enzyme has been reported as an indication of neuromuscular damage

associated with delayed neurotoxicity (Cisson and Wilson, 1983). CPK levels of all exposed female rabbit groups were comparable to the controls.

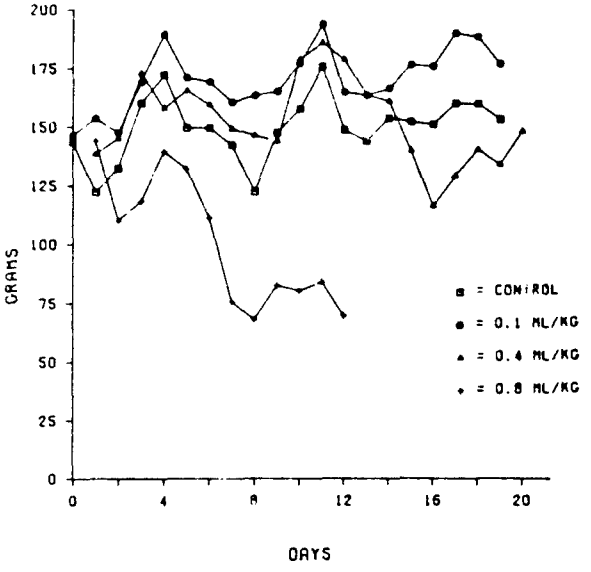


Figure 36. Effect of repeated dermal exposure to Durad MP280 on male rabbit food consumption.

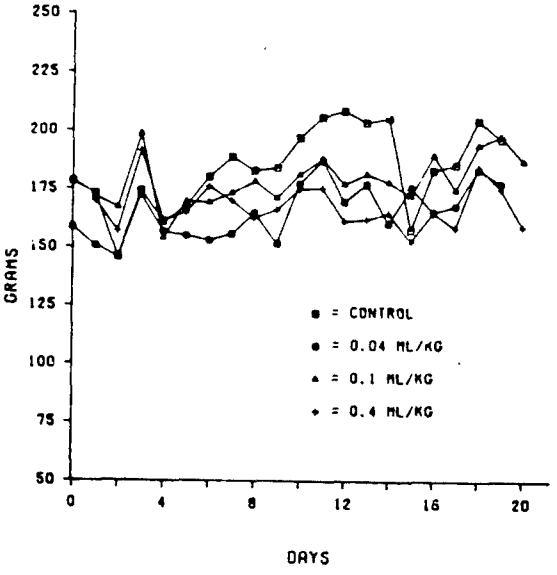


Figure 37. Effect of repeated dermal exposure to Durad MP280 on female rabbit food consumption.

TABLE 74. EFFECT OF REPEATED DERMAL EXPOSURE TO DURAD MP280 ON MALE RABBIT BLOOD PARAMETERS[a]

	Control	0.1 ml/kg	0.4 ml/kg	0.8 ml/kg
RBC ($x10^6$ cells/mm^3)	6.03 ± 0.16	6.04 ± 0.17	6.18 ± 0.23	5.97 ± 0.14
WBC ($x10^3$ cells/mm^3)	7.4 ± 0.3	7.5 ± 0.5	8.8 ± 0.8	8.4 ± 1.1
HCT (%)	39.5 ± 1.0	39.9 ± 0.7	40.4 ± 1.2	38.6 ± 1.1
HGB (g/dl)	13.6 ± 0.3	13.6 ± 0.2	13.8 ± 0.3	13.7 ± 0.3
MCV (μm^3)	65.5 ± 0.6	66.2 ± 0.8	65.5 ± 0.7	64.7 ± 0.6
MCH (pg)	22.6 ± 0.2	22.6 ± 0.4	22.3 ± 0.3	23.0 ± 0.2
MCHC (g/dl)	34.4 ± 0.3	34.2 ± 0.2	34.1 ± 0.3	35.6 ± 0.3[b]
Glucose (mg/dl)	127 ± 3	136 ± 3	129 ± 12	138 ± 16
BUN (mg/dl)	18.2 ± 1.0	19.3 ± 1.1	22.4 ± 4.4	27.8 ± 8.4
Creat.(mg/dl)	1.3 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	1.3 ± 0.1
T. Prot. (g/dl)	6.09 ± 0.11	6.13 ± 0.10	5.94 ± 0.17	5.27 ± 0.09[c]
Albumin (g/dl)	2.15 ± 0.04	2.18 ± 0.03	2.00 ± 0.05	1.80 ± 0.07[c]
Globulin (g/dl)	3.94 ± 0.09	3.95 ± 0.11	3.94 ± 0.15	3.40 ± 0.08[b]
A/G Ratio	0.55 ± 0.01	0.56 ± 0.02	0.51 ± 0.02	0.54 ± 0.02
SGOT (IU/L)	44 ± 6	41 ± 4	38 ± 4	55 ± 6
SGPT (IU/L)	39 ± 4	47 ± 5	35 ± 5	45 ± 7
Alk.Phos.(IU/L)	220 ± 18	213 ± 10	169 ± 23	80 ± 15[c]
Bili. (mg/dl)	0.4 ± 0.01	0.4 ± 0.00	0.4 ± 0.03	0.4 ± 0.09

[a] Values expressed as mean ± SE, N=6 to 9.
[b] Statistically different from control at $p < 0.05$.
[c] Statistically different from control at $p < 0.01$.

Organ weights measured at the conclusion of the 21-day exposure period are shown in Tables 76 and 77. The organ weights of the male rabbits exposed to Durad MP280 at concentrations up to 0.4 ml/kg were not statistically different from controls. Organ weights were not measured on the rabbits in the 0.8 ml/kg treatment group. Significant reductions in absolute heart and liver weight were noted in female rabbits exposed to Durad MP280 at 0.4 ml/kg. When the reduced body weight of this group was considered in the organ weight ratios, the heart weight was not significantly different from controls. The liver/body weight ratio of the 0.4 ml/kg female rabbit exposure group was significantly less than the control value. All organ weights of the 0.1 ml/kg and 0.04 ml/kg female rabbit exposure groups were comparable to controls.

TABLE 75. EFFECT OF REPEATED DERMAL EXPOSURE TO DURAD MP280 ON FEMALE RABBIT BLOOD PARAMETERS[a]

	Control	0.04 ml/kg	0.1 ml/kg	0.4 ml/kg
RBC ($x10^6$ cells/mm^3)	5.91 ± 0.12	5.94 ± 0.12	5.68 ± 0.13	5.78 ± 0.18
WBC ($x10^3$ cells/mm^3)	6.9 ± 1.0	7.6 ± 0.4	7.6 ± 0.4	8.6 ± 0.9
HCT (%)	39.1 ± 0.7	39.4 ± 0.7	37.8 ± 0.6	37.8 ± 1.0
HGB (g/dl)	13.7 ± 0.3	13.9 ± 0.2	13.1 ± 0.2	12.5 ± 0.9
MCV (um^3)	66.2 ± 0.6	66.4 ± 0.4	66.8 ± 1.3	65.5 ± 0.7
MCH (pg)	23.1 ± 0.3	23.4 ± 0.2	23.2 ± 0.5	21.6 ± 1.3
MCHC (g/dl)	34.9 ± 0.4	35.2 ± 0.2	34.8 ± 0.1	33.0 ± 1.9
Glucose (mg/dl)	125 ± 2	136 ± 2	128 ± 4	122 ± 5
BUN (mg/dl)	23.8 ± 1.3	24.6 ± 1.4	23.0 ± 1.1	32.0 ± 1.3[b]
Creatin.(mg/dl)	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.02	1.8 ± 0.1[c]
Tot.Prot.(g/dl)	6.04 ± 0.12	6.36 ± 0.15	6.70 ± 0.18[d]	6.56 ± 0.10
Albumin (g/dl)	2.24 ± 0.04	2.28 ± 0.05	2.29 ± 0.03	2.19 ± 0.03
Globulin (g/dl)	3.80 ± 0.10	4.09 ± 0.13	4.43 ± 0.18[d]	4.37 ± 0.10[d]
A/G Ratio	0.59 ± 0.02	0.56 ± 0.02	0.52 ± 0.02	0.50 ± 0.02[c]
SGOT (IU/L)	51 ± 15	41 ± 5	48 ± 4	36 ± 3
SGPT (IU/L)	56 ± 16	40 ± 6	39 ± 3	38 ± 3
Alk.Phos.(IU/L)	208 ± 25	223 ± 22	182 ± 11	200 ± 16
Bilirub.(mg/dl)	0.4 ± 0.02	0.4 ± 0.01	0.3 ± 0.02	0.3 ± 0.02
Creatin. phosphok. (IU/L)	746 ± 179	457 ± 88	729 ± 120	789 ± 199

[a] Values expressed as mean ± SE, N=7 to 10.
[b] Statistically different from control at $p < 0.001$.
[c] Statistically different from control at $p < 0.01$.
[d] Statistically different from control at $p < 0.05$.

In summary, the cumulative effects of 15 repeated dermal exposures of male rabbits to Durad MP280 at doses of 0.4 ml/kg or 0.8 ml/kg included reduced weight gain, reduced food consumption, and death. Female rabbits treated at 0.4 ml/kg also demonstrated reduced weight gain but food consumption rates were not significantly different from controls. Tissues taken for microscopic examination are being processed. The results of these examinations will be reported in future annual reports.

TABLE 76. EFFECT OF REPEATED DERMAL EXPOSURE TO DURAD MP280 ON MALE RABBIT ORGAN WEIGHT

	Control	0.1 ml/kg	0.4 ml/kg
Body wt (kg)	2.99 ± 0.06	2.96 ± 0.07	2.81 ± 0.11
Heart wt (g)	8.58 ± 0.82	8.78 ± 0.79	10.55 ± 1.28
Heart/100 g body wt	0.29 ± 0.03	0.30 ± 0.03	0.38 ± 0.06
Testes wt (g)	3.44 ± 0.28	3.49 ± 0.22	2.97 ± 0.19
Testes/100 g body wt	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Liver wt (g)	102.11 ± 7.50	101.47 ± 3.98	96.58 ± 6.22
Liver/100 g body wt	3.41 ± 0.22	3.44 ± 0.17	3.43 ± 0.15
Spleen wt (g)	1.22 ± 0.13	1.41 ± 0.33	1.14 ± 0.12
Spleen/100 g body wt	0.04 ± 0.004	0.05 ± 0.01	0.04 ± 0.001
Kidney wt (g)	16.56 ± 1.01	18.36 ± 1.18	17.07 ± 1.06
Kidney/100 g body wt	0.56 ± 0.04	0.62 ± 0.04	0.61 ± 0.04

TABLE 77. EFFECT OF REPEATED DERMAL EXPOSURE TO DURAD MP280 ON FEMALE RABBIT ORGAN WEIGHT[a]

	Control	0.04 ml/kg	0.1 ml/kg	0.4 ml/kg
Body wt (kg)	3.00 ± 0.08	2.85 ± 0.05	2.87 ± 0.07	2.68 ± 0.06[b]
Heart wt (g)	9.66 ± 0.87	7.84 ± 0.66	7.30 ± 0.47	7.04 ± 0.41[b]
Heart/100 g body wt	0.32 ± 0.03	0.27 ± 0.02	0.26 ± 0.02	0.26 ± 0.01
Liver wt (g)	105.55 ± 6.45	102.44 ± 4.70	99.03 ± 3.91	78.48 ± 2.92[c]
Liver/100 g body wt	3.50 ± 0.16	3.59 ± 0.14	3.44 ± 0.09	2.93 ± 0.11[b]
Spleen wt (g)	1.19 ± 0.10	1.29 ± 0.10	1.76 ± 0.20	1.32 ± 0.24
Spleen/100 g body wt	0.04 ± 0.003	0.05 ± 0.003	0.06 ± 0.01	0.05 ± 0.01
Kidney wt (g)	16.28 ± 0.68	15.55 ± 0.46	16.94 ± 0.55	14.58 ± 0.74
Kidney/100 g body wt	0.54 ± 0.02	0.55 ± 0.01	0.59 ± 0.03	0.54 ± 0.02

[a] Values expressed as mean ± SE, N=9 or 10.
[b] Significantly different from control at $p < 0.05$.
[c] Significantly different from control at $p < 0.01$.

THE ACUTE IRRITATION AND SENSITIZATION POTENTIAL OF JP-4, JP-7, JP-8, AND JP-TS JET FUELS

The THRU has conducted inhalation studies on a number of hydrocarbon fuels used by the Air Force and Navy. These fuels include JP-4 (Petroleum and Shale derived), JP-5 (Petroleum and Shale derived), JP-7, JP-8, JP-10, JP-TS, and Diesel Fuel Marine (Petroleum and Shale derived). Along with the inhalation tests, five of these fuels have been tested for skin and eye irritation potential as well as sensitization response. JP-4 (Petroleum and Shale derived), JP-7, JP-8, and JP-TS were not previously subjected to irritation and sensitization tests. To complete the information necessary for a comparison of all of the fuels, irritation and sensitization tests were conducted on JP-4 (Petroleum and Shale derived), JP-7, JP-8, and JP-TS.

Methods

Animals - New Zealand white rabbits (2-3 kg) were used for the eye and skin irritation tests. Hartley albino guinea pigs (200-400 g) were used for sensitization testing.

Materials - The fuels tested were complex mixtures of aliphatic and aromatic hydrocarbon compounds. Various additives including antioxidant and icing inhibitors were added to the fuels during manufacturing to comply with military specifications.

Eye Irritation - One-tenth milliliter of the undiluted test material was applied to one eye of each of nine albino rabbits. The opposite eye was untreated and served as a control. The eyes of the test animals were examined with fluorescein stain prior to use to ensure the absence of lesions or injury. The treated eye of six rabbits remained unwashed while the remaining three rabbits received test material and then had the treated eye flushed for one minute with lukewarm water starting no sooner than 20-30 seconds after instillation. Examinations for gross signs of eye irritation were made at 1, 2, 3, 4, and 7 days following application. Scoring of irritative effects was according to the method of Draize (1959) in which corneal, iris, and conjunctival effects were scored separately.

Skin Irritation - A patch-test method was utilized to determine the degree of primary skin irritation of intact and abraded skin of albino rabbits.

Six rabbits were clipped of all possible hair on the back and flanks 24 hours prior to exposure to allow for recovery of the skin from any abrasion resulting from the clipping. One of the two test areas on the back was abraded with a hypodermic needle through the stratum corneum, but not sufficiently deep to disturb the dermis or to produce bleeding.

Undiluted test material was applied in the amount of 0.5 ml to the designated patch area and covered by a 1-inch square of surgical gauze two single layers thick. The gauze patches were held in place with strips of surgical adhesive tape. The entire area was covered with polyethylene plastic wrap and secured with more surgical adhesive tape. These patches remained in place on the rabbits for 24 hours. After 24 hours, the wrap and patches were carefully removed, and the test areas were evaluated for irritation using the Draize (1959) table as a reference standard. A combined score was recorded. Examinations were also made at 72 hours (48 hours after the first reading). Scoring continued thrice weekly until injury subsided.

Skin Sensitization - Ten albino guinea pigs, six to eight weeks of age, were used. Each material was tested for primary irritation on three guinea pigs by application to the clipped flank. Observation was made at 24 hours for signs of irritation. If the test material was irritating to the guinea pig skin, dilutions were made.

An area on the back of each animal directly above the forelegs was clipped with electric clippers and chemically depilated with a commercial depilatory on the morning of the first insult exposure. Groups consisted of 10 animals. Test solution, 0.1 ml at each application, was applied to this area on a 1/2 x 1/2 inch cotton gauze square, covered with dental dam and held in place with adhesive tape. The first insult patch remained in place for two days, was removed, and a second application of 0.1 ml was made. Two days later, this patch was removed, a total of 0.2 ml of a 50% aqueous dilution of Freund's adjuvant per animal was injected intradermally, using 2 or 3 points adjacent to the insult site, then a new patch of 0.1 ml of the test material was applied. On the third day after this application, the patch was removed and a new patch of 0.1 ml of the material was applied. The last patch was removed two days later, and the animals were allowed to rest for two weeks. Each time the insult patches were removed, the condition of the skin at the application site was evaluated. When the last patch was removed, the toes of the hind feet of each animal were taped to prevent the animal from scratching the irritated area.

After the two-week rest period, both flanks of the animals were clipped and challenged on one side with the test solution. The challenge applications were not occluded. The skin response at these sites was evaluated at 24 and 48 hours after application. Any animal showing measureable erythema and/or edema at the test solution challenge site was rated as a positive responder.

In scoring the Maguire Test, the important statistic is frequency of the reaction. The following table is used to classify test materials as to sensitization potential.

Sensitization Rate (%)	Grade
10	I Weak
20 - 30	II Mild
40 - 60	III Moderate
70 - 80	IV Strong
90 - 100	V Extreme

Results

Eye Irritation - None of the fuels tested produced any sign of irritation in either washed or unwashed eyes.

Skin Irritation - The results of the primary skin irritation tests are shown in Table 78. JP-7 produced the greatest degree of skin irritation when measured by the patch test method. Erythema and edema were evident in five of the six rabbits examined at 24 hours. Similar effects were noted at 72 hours. JP-4 derived from either Petroleum or Shale sources produced identical reaction patterns, no irritation at 24 hours with moderate erythema and edema at 72 hours. Shale JP-4 produced more intense reactions than Petroleum JP-4. Rabbits treated with JP-8 and JP-TS developed mild erythema after 24 hours of contact. The erythema was intensified at 72 hours. Neither JP-8 or JP-TS produced edema. Examination at 1-week postapplication revealed eschar or epidermal exfoliation in many of the rabbits treated with the fuels. Eschar formation was most intense in the rabbits treated with JP-7. Erythema and edema had generally subsided in all rabbits examined at 1-week postapplication.

Skin Sensitization - The results of the sensitization tests are shown in Table 79. The animals treated with Petroleum JP-4 exhibited no sensitization response. In contrast to this, Shale JP-4 demonstrated responses indicative of a mild to moderate

sensitizer. Erythema was present in the majority of the animals examined at 24 hours postchallenge. The frequency of response was greatly reduced when the 48 hour examination was conducted.

TABLE 78. PRIMARY SKIN IRRITATION TEST RESULTS: JP-4, JP-7, JP-8, AND JP-TS

Fuel	P.I. Score[a]	Symptom		
		24 Hours	72 Hours	1 Week
JP-4 (Petroleum)	1.04	No Irritation	Moderate erythema, no edema	Mild exfoliation, mild erythema, no edema
JP-4 (Shale)	2.13	No Irritation	Moderate erythema, mild edema	Mild erythema, mild edema
JP-7	2.25	Moderate erythema, slight edema	Moderate erythema, slight edema	Moderate to severe eschar formation
JP-8	1.29	Slight erythema, no edema	Mild to moderate erythema no edema	Mild to moderate eschar formation, no erythema, no edema
JP-TS	1.08	Slight erythema, no edema	Mild to moderate erythema, no edema	Mild eschar formation, no erythema, no edema

[a] Primary Index Score = $\frac{\text{Total Score}}{\text{No. Rabbits x No. sites x No. observations}}$

JP-7, JP-8, and JP-TS produced erythema in a few of the animals tested. Intensity of the erythematous reactions and frequencies of responses were less at 48 hours when compared to 24 hours. These materials show weak to mild sensitization potential.

TABLE 79. SKIN SENSITIZATION TEST RESULTS: JP-4, JP-7, JP-8, AND JP-TS

Material	Number Tested	Positive Responses 24 Hrs.	Positive Responses 48 Hrs.
JP-4 (Petroleum)	9	0	0
JP-4 (Shale)	10	8	3
JP-7	10	4	2
JP-8	10	3	1
JP-TS	10	2	1

On examination of the sensitization test results for 10 fuels (5 reported here and 5 reported as long as 5 years ago) we found that all showed positive results except JP-4 Petroleum. The 5 fuels tested in the past are DFM Petroleum and Shale, JP-5 Petroleum and Shale, and JP-10.

During the testing of JP-7, observations of reactions caused suspicion that the use of this fuel in an undiluted form caused primary skin irritation which would mask any sensitization response. Because of this uncertainty it was decided to repeat the sensitization test of JP-7, but in a diluted form.

A comprehensive report of the results of primary eye and skin irritation and sensitization tests of 10 fuels will be made in the next annual report.

THE ACUTE TOXICITY AND SUBCHRONIC DERMAL TOXICITY OF ANTIMONY THIOANTIMONATE LUBRICANTS

The U. S. Navy is currently using a graphite based grease to lubricate aircraft catch cables on carriers. These cables are stretched across the flight deck and are snagged by the arresting hook of incoming aircraft. This graphite lubricant is difficult to remove during clean-up and the slippery deck creates a safety hazard. Therefore the Navy is evaluating a new lubricant that

has a basic composition of 3-5% antimony thioantimonate (ATA) in calcium (Ca) cup grease #3. This lubricant is undergoing performance evaluation at the Naval Air Engineering Center.

As part of the investigation of the lubricant the Navy requested that the THRU conduct a series of acute and subchronic toxicity studies. ATA was used as the technical material (dry, burnt-orange powder) for intraperitoneal toxicity and eye irritation tests. Skin sensitization ests were conducted with ATA technical material and the full 5% formulation. Ca cup grease was also tested for sensitization potential. Investigations of the acute intraperitoneal toxicity, eye irritation, and skin sensitization properties of the ATA technical material as well as investigations of the skin sensitization of the ATA Ca cup grease have been completed and were reported in the 1983 Annual Report (MacEwen and Vernot, 1983). The acute intraperitoneal LD_{50} values of ATA were found to be 445 and 568 mg/kg to male and female rats, respectively. ATA technical was found to cause eye irritation that was reversed within 13 days posttreatment. ATA technical grade demonstrated no sensitization potential; however, the full formulation and Ca cup grease were shown to be sensitizing agents. ATA Technical caused no deaths in male or female rats when administered orally at a dose of 5 g/kg or in rabbits when given dermal doses of 2 g/kg (Wolfe 1981, 1981a). Slight reversible eye irritation (Latven, 1981) and slight skin irritation (Latven, 1981a) were the result of ATA applications. A literature search revealed numerous publications on the human toxicity of various antimony compounds used as parasiticides. The primary effects reported were cardiac, primarily changes in T wave configuration (Schroeder et al., 1946. There was also some indication of SGOT elevations (Waye et al., 1962).

NMRI/TD also requested that the THRU evaluate the subchronic dermal toxicity of the full formulation. A 90-day repeated dermal toxicity test was developed for this investigation. This report presents the results of the 90-day test with male rabbits.

Materials

The test materials supplied by the Navy for study are described below:

FULL FORMULATION 5% ANTIMONY THIOANTIMONATE IN CALCIUM CUP GREASE

Manufacturer:	Pennwalt Corporation
Batch No.:	565D-5-1
Chemical Family:	Calcium Soap-thickened petroleum hydrocarbons with 5% antimony thioantimonate

0.5% ANTIMONY THIOANTIMONATE IN CALCIUM CUP GREASE

Manufacturer:	Pennwalt Corporation
Chemical Family:	Calcium Soap - thickened petroleum hydrocarbons with 0.5% antimony thioantimonate

CALCIUM CUP GREASE NO. 3

Manufacturer:	Cooke Industrial Lubricants, Inc.
Boiling Pt., °F:	545
Vapor Pressure (mmHg):	<0.01
Specific Gravity:	0.9
Chemical Family:	Calcium Soap-thickened petroleum hydrocarbons

Methods

Groups of 10 male New Zealand white rabbits weighing between 2-3 kg received occluded applications on weekdays (normal working days) for 13 consecutive weeks. Occlusion lasted for 6 hours daily. Hair was clipped from the backs of the animals as necessary. The test materials were weighed onto 4" x 4" gauze patches which were then placed over the clipped areas. The entire trunk of the animal was then wrapped with an impervious polyethylene plastic wrap held in place by 2-inch surgical tape. After removal of the wrapping, the skin was wiped with paper towels in order to remove excess material.

Initially the exposed skin of 5 animals from each group was abraded weekly. However, this practice was terminated when the animals began to exhibit skin irritation. Dermal irritation scores according to the method of Draize (1959) were recorded daily for each animal prior to application of the test material.

The treatment groups received Ca cup grease (vehicle control), ATA full formulation (5% ATA in Ca cup grease), or a 1 to 10 dilution of the full formulation (0.5% ATA in Ca cup grease). An untreated group was also maintained as a negative control. All materials were applied at a dose of 2 g test material/kg of body weight. The 2 g dose level was selected since it is the upper level cut-off value specified in EPA acute dermal guidelines.

Body weights were obtained daily just prior to dosing until approximately 3 weeks when the frequency of weighing was reduced to twice weekly. Prior to the onset of the study, blood was drawn for hematologic and clinical chemistry pretreatment determinations. Blood was also taken from 5 animals in each of the negative control, 0.5% ATA, and 5% ATA groups after approximately 4 weeks. All animals were bled at study termination. Preexposure electrocardiograms (ECG) were conducted on all animals and were repeated after approximately 3 and 7 weeks on test and again just prior to the test conclusion. Food consumption was measured on week days during the entire course of the study. Food and water were available ad libitum.

All surviving animals were necropsied at study termination and the following tissues taken for histologic evaluation:

Liver	Thyroid
Adrenals	Brain
Kidney	Skin
Heart	Gross lesions
Testes	

Heart, kidneys, liver, and brain weights were measured at necropsy. The hearts of 2 rabbits from the negative control and 5% ATA groups were perfused in situ in preparation for electron microscopic examination.

Results

No deaths occurred in male rabbits exposed to either of the ATA formulations. One rabbit from the vehicle control group died after approximately one month on test. Gross necropsy revealed no findings of significance. Another rabbit from the vehicle control exhibited severe weight loss and reduced food consumption. Upon physical examination several large subcutaneous

masses were palpated. The decision was made to sacrifice this animal. Upon necropsy cultures were taken from the abcesses. These cultures indicated Pasturella multocida.

Within a few weeks of study initiation, the majority of rabbits from all treatment groups, including the vehicle control group, began to exhibit skin lesions. These appeared to be edematous in nature followed by a thickening of the epidermis with some minor exfoliation. The degree of severity of these lesions remained constant throughout the remainder of the test.

Heart, liver, kidney, and brain were weighed at the time of sacrifice. The mean organ and mean organ to body weight ratios are presented in Table 80. The kidney weights of animals from the 5% ATA group were different from those of the vehicle control group, but not the negative control group at the 0.05 level of significance. The difference was not significant when relative organ weight ratios were compared.

A summary of hematologic and clinical chemistry parameters from the preexposure, 4-week interim and postexposure bleedings is presented in Table 81. Creatinine and BUN values at termination in the higher dose groups were statistically different from control. Whether these differences and the decreased kidney weight are biologically significant cannot be determined until histopathology results are obtained.

Body weight and food consumption graphs are presented in Figures 38 and 39, respectively. The vehicle control, 0.5% ATA, and 5% ATA groups demonstrated decreased weight gain when compared to the negative control group. At no time during the study were the differences between the vehicle control or 0.5% ATA group significantly different from the negative control group. The differences in body weight between the 5% ATA group and negative control group were intermittently significant at $p < 0.05$. The 5% ATA group body weights were also significantly depressed when compared to the vehicle control group at 2 points. The food consumption data presented in Figure 39 demonstrated such a high degree of variability that no meaningful analysis was possible. The results of the electrocardiogram examinations are summarized in Table 82. A linear contrast multivariate analysis of variance was performed on these data. All variables with the exception of the PR interval demonstrated parallelism for all treatments, with no significant dose effect and a significant time effect. Since the PR interval demonstrated non-parallelism, separate

comparisons of the means at each time period were conducted. The 5% ATA group was found to have a slight but significantly increased PR interval at the 7-week interim examination.

Examination of the tissues taken at necropsy is not complete. Without these findings definite conclusions cannot be drawn. However, of the parameters examined to date the only significant effect of repeated dermal contact with ATA lubricants was a decrease in body weight gain. This decrease appeared to be dose related and was intermittently significant for the 5% ATA group.

TABLE 80. MEAN ORGAN WEIGHT VALUES[a] OF MALE RABBITS EXPOSED DERMALLY FOR 90 DAYS TO ATA MATERIALS

	Negative Control	Ca Cup Grease Vehicle Control	0.5% ATA/ Ca Cup Grease	5% ATA/ Ca Cup Grease
Heart				
wt (g)	9.0 ± 0.87	8.9 ± 0.88	9.6 ± 1.18	9.4 ± 1.02
Organ/body wt x 100	0.2 ± 0.03	0.2 ± 0.02	0.3 ± 0.03	0.3 ± 0.03
Liver				
wt (g)	105.5 ± 5.1	110.2 ± 6.1	110.3 ± 4.7	105.5 ± 6.7
Organ/body wt x 100	2.7 ± 0.1	3.0 ± 0.13	3.2 ± 0.1	3.1 ± 0.11
Kidney				
wt (g)	19.2 ± 0.4	19.9 ± 1.4	19.1 ± 1.1	15.5 ± 1.0[b]
Organ/body wt x 100	0.5 ± 0.02	0.6 ± 0.03	0.6 ± 0.03	0.5 ± 0.01
Brain				
wt (g)	9.3 ± 0.19	9.7 ± 0.21	9.1 ± 0.55	9.1 ± 0.14
Organ/body wt x 100	0.2 ± 0.01	0.3 ± 0.01	0.3 ± 0.02	0.3 ± 0.02

[a] Mean ± SE, N=7 to 10.
[b] Significantly different from vehicle control at $p < 0.05$.

TABLE 81. EFFECT OF REPEATED DERMAL EXPOSURE TO ATA MATERIALS ON MALE RABBIT BLOOD PARAMETERS[a]

	Negative Control	Ca Cup Grease Vehicle control	0.5% ATA/Ca Cup Grease	5% ATA/Ca Cup Grease
WBC (x 10^3 cells/mm^3)				
Preexposure	7.5 ± 0.6	10.0 ± 1.6	9.5 ± 1.2	8.2 ± 0.9
4 Week Interim	9.7 ± 1.0	7.1 ± 0.7	---	8.3 ± 0.3
Termination	7.9 ± 0.5	7.0 ± 0.7	8.9 ± 0.6	8.2 ± 0.6
RBC (x 10^6 cells/mm^3)				
Preexposure	5.1 ± 0.2	5.5 ± 0.1	5.3 ± 0.1	5.3 ± 0.2
4 Week Interim	5.9 ± 0.2	5.6 ± 0.2	---	6.0 ± 0.2
Termination	6.5 ± 0.1	6.2 ± 0.2	6.5 ± 0.1	6.4 ± 0.1
HGB (g/dl)				
Preexposure	11.4 ± 0.4	12.2 ± 0.3	11.7 ± 0.2	11.8 ± 0.2
4 Week Interim	13.0 ± 0.3	12.3 ± 0.4	---	13.1 ± 0.3
Termination	15.0 ± 0.2	13.9 ± 0.4[b]	14.4 ± 0.2	14.3 ± 0.1
HCT (%)				
Preexposure	33.4 ± 1.1	35.9 ± 0.8	34.8 ± 0.9	34.2 ± 1.2
4 Week Interim	38.7 ± 1.0	36.9 ± 1.2	---	39.0 ± 1.2
Termination	42.3 ± 0.7	39.3 ± 1.2	41.4 ± 0.7	41.6 ± 0.6
MCV (μm^3)				
Preexposure	66.1 ± 1.6	65.8 ± 0.6	65.2 ± 0.7	65.1 ± 1.1
4 Week Interim	66.1 ± 0.5	66.1 ± 1.5	---	64.8 ± 1.5
Termination	64.8 ± 0.5	63.6 ± 0.3	64.1 ± 0.4	64.9 ± 0.8
MCH (pg)				
Preexposure	22.5 ± 0.5	22.3 ± 0.3	21.9 ± 0.3	22.6 ± 0.8
4 Week Interim	22.2 ± 0.2	22.1 ± 0.5	---	21.8 ± 0.5
Termination	23.0 ± 0.3	22.5 ± 0.2	22.3 ± 0.4	22.3 ± 0.3
MCHC (g/dl)				
Preexposure	34.1 ± 0.3	34.0 ± 0.4	33.6 ± 0.3	34.7 ± 1.0
4 Week Interim	33.6 ± 0.2	33.4 ± 0.3	---	33.7 ± 0.3
Termination	35.5 ± 0.2	35.4 ± 0.2	34.8 ± 0.4	34.4 ± 0.3[b]
Glucose (mg/dl)				
Preexposure	218 ± 38	220 ± 39	179 ± 15	164 ± 5
4 Week Interim	139 ± 6	150 ± 2	---	156 ± 16
Termination	119 ± 2	122 ± 3	132 ± 3	133 ± 3
Bun (mg/dl)				
Preexposure	---	---	---	---
4 Week Interim	17.4 ± 1.1	17.6 ± 0.9	---	18.2 ± 1.0
Termination	20.0 ± 1.0	18.9 ± 1.2	23.2 ± 1.7	24.4 ± 1.4[c]
Creatinine (mg/dl)				
Preexposure	0.95 ± 0.05	0.96 ± 0.07	0.91 ± 0.04	0.99 ± 0.04
4 Week Interim	1.1 ± 0.07	1.0 ± 0.03	---	1.1 ± 0.05
Termination	1.4 ± 0.06	1.2 ± 0.08	1.5 ± 0.10	1.7 ± 0.08[bc]
Total Protein (g/dl)				
Preexposure	5.6 ± 0.2	6.3 ± 0.2[b]	5.9 ± 0.1	5.9 ± 0.1
4 Week Interim	5.7 ± 0.3	5.9 ± 0.2	---	5.6 ± 0.2
Termination	6.3 ± 0.1	6.1 ± 0.1	6.3 ± 0.1	6.2 ± 0.1
Albumin (g/dl)				
Preexposure	2.1 ± 0.05	2.1 ± 0.06	2.1 ± 0.06	2.1 ± 0.04
4 Week Interim	1.8 ± 0.07	1.9 ± 0.02	---	1.8 ± 0.07
Termination	1.9 ± 0.06	1.9 ± 0.03	1.9 ± 0.06	1.9 ± 0.02
Globulin (g/dl)				
Preexposure	3.6 ± 0.1	4.2 ± 0.2[b]	3.8 ± 0.1	3.8 ± 0.1
4 Week Interim	3.8 ± 0.2	4.0 ± 0.2	---	4.0 ± 0.2
Termination	4.1 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.3 ± 0.1
A/G Ratio				
Preexposure	0.6 ± 0.01	0.5 ± 0.03	0.6 ± 0.02	0.6 ± 0.02
4 Week Interim	0.5 ± 0.01	0.5 ± 0.03	---	0.5 ± 0.01
Termination	0.4 ± 0.01	0.4 ± 0.01	0.[illegible] ± 0.01	0.4 ± 0.004
SGOT (IU/l)				
Preexposure	46 ± 6	55 ± 8	5[illegible] ± 8	51 ± 10
4 Week Interim	25 ± 2	32 ± 5	---	37 ± 3[b]
Termination	47 ± 6	56 ± 11	89 ± 12[b]	85 ± 17
SGPT (IU/l)				
Preexposure	46 ± 6	55 ± 6	53 ± 6	51 ± 10
4 Week Interim	39 ± 4	40 ± 3	---	41 ± 5
Termination	51 ± 5	68 ± 19	80 ± 14	55 ± 4
Alk. Phos. (IU/l)				
Preexposure	167 ± 8	179 ± 21	170 ± 16	213 ± 22
4 Week Interim	203 ± 18	209 ± 31	---	232 ± 30
Termination	119 ± 12	112 ± 15	135 ± 20	124 ± 19
Bilirubin (mg/dl)				
Preexposure	0.40 ± 0.03	0.46 ± 0.02	0.43 ± 0.02	0.48 ± 0.04
4 Week Interim	0.11 ± 0.02	0.13 ± 0.03	---	0.14 ± 0.02
Termination	0.10 ± 0.00	0.10 ± 0.00	0.13 ± 0.02	0.14 ± 0.02

[a] Values expressed as mean ± SE (N).
[b] Different from the negative control at the 0.05 level.
[c] Different from the vehicle control at the 0.05 level.

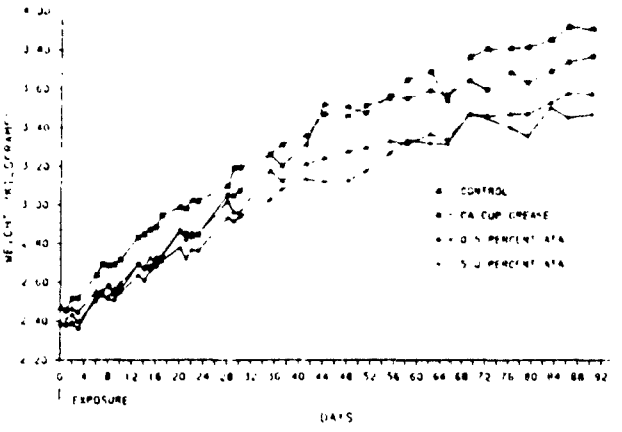


Figure 38. Body weights of male rabbits during 90-day dermal exposure to ATA.

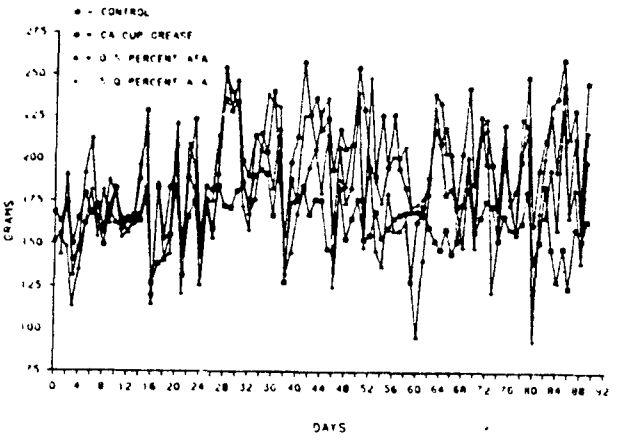


Figure 39. Food consumption of male rabbits during 90-day dermal exposure to ATA.

A 90-day repeated dose dermal toxicity test is being conducted with female rabbits. The results of these tests will be presented in a future annual report.

TABLE 82. SUMMARY OF ELECTROCARDIOGRAM RESULTS FROM MALE RABBITS EXPOSED TO ATA MATERIALS[a]

Parameter	Negative Control	Ca Cup Grease Vehicle Control	0.5% ATA/ Ca Cup Grease	5% ATA/ Ca Cup Grease
P Interval (msec)				
Preexposure	24 ± 2	32 ± 5	28 ± 3	24 ± 2
3 Week	30 ± 2	32 ± 3	29 ± 1	34 ± 2
7 Week	30 ± 2	32 ± 2	33 ± 2	35 ± 3
Termination	29 ± 2	33 ± 3	30 ± 3	26 ± 2
PR Interval (msec)				
Preexposure	64 ± 4	61 ± 3	55 ± 4	53 ± 3
3 Week	67 ± 3	65 ± 4	64 ± 3	64 ± 4
7 Week	64 ± 3	64 ± 2	65 ± 4	68 ± 6[b]
Termination	59 ± 6	71 ± 4	66 ± 4	59 ± 3
QRS Interval (msec)				
Preexposure	29 ± 1	33 ± 5	34 ± 3	34 ± 2
3 Week	38 ± 3	41 ± 5	57 ± 6	48 ± 7
7 Week	33 ± 2	38 ± 4	35 ± 2	38 ± 3
Termination	32 ± 2	31 ± 3	31 ± 2	32 ± 3
QT Interval (msec)				
Preexposure	154 ± 6	143 ± 7	143 ± 14	149 ± 8
3 Week	144 ± 6	141 ± 7	143 ± 6	135 ± 3
7 Week	143 ± 3	149 ± 4	139 ± 6	151 ± 6
Termination	144 ± 3	139 ± 4	123 ± 6	141 ± 6
P Amplitude (mv)				
Preexposure	47 ± 4	54 ± 8	51 ± 7	53 ± 8
3 Week	61 ± 7	64 ± 9	56 ± 6	54 ± 9
7 Week	39 ± 4	51 ± 5	46 ± 4	53 ± 6
Termination	47 ± 2	54 ± 6	41 ± 6	43 ± 6
T Amplitude (mv)				
Preexposure	250 ± 30	300 ± 40	260 ± 50	200 ± 30
3 Week	180 ± 30	270 ± 70	150 ± 30	130 ± 20
7 Week	140 ± 20	180 ± 30	160 ± 30	140 ± 20
Termination	130 ± 10	110 ± 20	110 ± 20	110 ± 10
Heart Rate (BPM)				
Preexposure	209 ± 13	210 ± 15	206 ± 12	199 ± 17
3 Week	231 ± 11	246 ± 15	240 ± 11	244 ± 17
7 Week	228 ± 10	209 ± 11	209 ± 12	194 ± 16
Termination	196 ± 13	178 ± 11	210 ± 11	199 ± 20

[a] Mean ± SE, N=6 to 10.
[b] Different from the negative control at the 0.05 level.

ACUTE TOXICITY STUDIES ON AIR FORCE HYDRAULIC FLUIDS

The Toxic Hazards Research Unit was requested to evaluate the acute toxicity of two hydraulic fluids, MLO 82-233 and MLO 82-585. These two hydraulic fluids, currently in use, meet the Air Force specifications MIL-H-83282 and MIL-H-5606, respectively.

Because of the possibility of exposure of military and civilian personnel to these triarylphosphate containing mixtures during manufacturing, processing, and transportation, the Air Force wanted to have the toxic hazards of the two hydraulic fluids defined. MIL-H-5606 specifies a petroleum base hydraulic fluid intended for use in aircraft, missile, and ordinance while MIL-H-83282 defines a fire-resistant, synthetic hydrocarbon base hydraulic fluid for aircraft use. Both hydraulic fluids contain varying amounts of tricresylphosphate (TCP), the amount of which is not limited in the specifications although the ratio of the ortho isomer (TOCP) to TCP is defined.

Tests conducted include acute oral, dermal, and inhalation toxicity as well as eye and skin irritation and sensitization. Since both fluids contain TCP, acute neurotoxic testing was also performed.

Many organophosphorus compounds have been found to cause delayed neurotoxic effects in man (Doull et al., 1979). A single exposure to a neurotoxic organophosphorus compound has been reported capable of producing axonal damage after a delay of eight to ten days. Low level nerve injury may occur in humans after chronic exposure to these compounds. Similar neurotoxic effects have been demonstrated in adult chickens and cats after exposure to triorthocresylphosphate (TOCP) by Beresford and Glees (1963).

Materials and Methods

Animals - Male and female New Zealand white rabbits were obtained from Price Rabbitry, New Carlisle, Ohio. Male and female Sprague-Dawley rats were purchased from Charles River Breeding Laboratories, Wilmington, Mass. The male and female Hartley derived guinea pigs were obtained from Murphy Breeding Laboratories, Plainfield, Indiana.

Leghorn hens (Gallus domesticus, Carey-Nick; 300-320 hybrid) five to seven months of age and weighing between 1.2 and 3.0

kilograms were purchased from Carey Farms, LaRue, Ohio. The debeaked hens were group housed in three by six foot pens to allow for freedom of movement.

Test Materials

Properties of petroleum base stock MIL-H-5606 (MLO 82-585):

Property	Limits
Pour point (max.)	-60°C
Flash point (min.)	82°C
Acid or base No. (max.)	0.10
Color, ASTM Std. (max.)	No. 1

This compound has as its base stock naphthenic type petroleum oil and may contain the following additives: viscosity - temperature coefficient improvers, oxidation inhibitors, antiwear agents, and red dye. The specific formula for the finished hydraulic fluid is proprietary.

Properties of the synthetic hydrocarbon base stock, MIL-H-83282 (MLO 82-233):

Property	Limits
Pour Point (max.)	-54°C
Flash Point (min.)	204.4°C
Acid or Base No. (max.)	0.10
Color, Saybolt (min.)	+30
Fire Point	246.1°C

This fluid has a base stock of a synthetic hydrogenated poly-alphaolefin with a nominal $C_{30}H_{62}$ formula. The main additive, approximately 30%, is an ester and may be either a polyolester or a diester. The fluid also contains oxidation inhibitors, antiwear agents, blending fluids, and red dye. The exact composition of the finished fluid is proprietary.

Both hydraulic fluids were analyzed for o-cresol content. Following a base hydrolysis of the phosphate esters in a Parr Bomb, the phenolic portion was extracted with diethyl ether. The extract was analyzed by a gas chromatograph/mass spectrometer (GC/MS).

The saponified and extracted MLO 82-233 sample showed no o-cresol within the sensitivity of the GC/MS, which is 15 ppm. However, a slight response was noted with the MLO 82-585 sample at the o-cresol retention time of 6.5 minutes (Figure 40). The peak at seven minutes was identified as m-cresol on the basis of mass spectral data and previous experience. The relationship of the two peaks of the total ionization scan compared to that of the 108 atomic mass unit scan (amu) indicate that there may be some o-cresol present in the sample but its concentration would be extremely low.

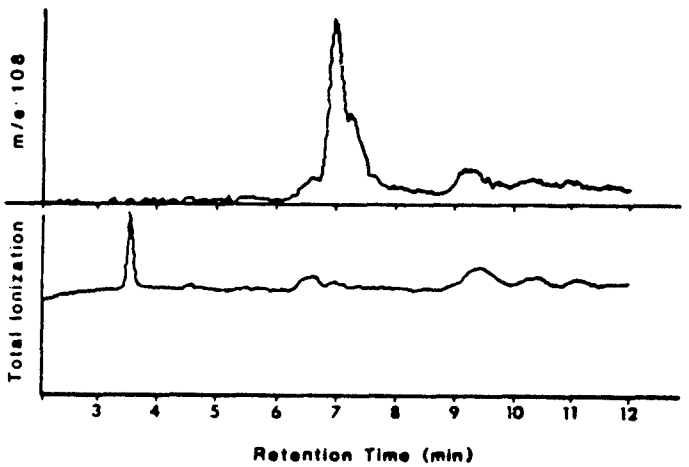


Figure 40. A comparison of total ion and single ion (m/e 108) GC/MS chromatograms of MLO 82-585.

Oral Toxicity - The test materials were administered dissolved in corn oil, using glass syringes equipped with ball-tipped oral dosing needles. A dose volume of 0.5 ml/100 grams of body weight was used. Test animals were male and female Sprague-Dawley rats weighing 200-300 grams and 150-250 grams, respectively. Five rats of each sex were dosed at 5 ml/kg of body weight. The animals were fasted overnight prior to dosing.

Animals were observed frequently on the day of dosing and twice daily during the 14-day observation period. Visible signs of toxicity were recorded. Body weights were obtained at the time of dosing and at 1, 2, 4, 7, 10, and 14 days posttreatment.

Dermal Toxicity - Male and female albino New Zealand rabbits weighing between 2 and 3 kilograms were used. The rabbits were clipped as closely as possible with an Oster® clipper equipped with surgical blades and a vacuum attachment.

Five animals of each sex were dosed at 2 ml/kg of body weight. The liquid material was applied undiluted to the back of the rabbit and divided as equally as possible between the two sides. The dose was kept in place by applying 8 ply gauze patches over the liquid on each side of the back. A patch of clear plastic wrap was then applied over the entire clipped back area and elastoplast tape was used to wrap the entire midsection of the rabbit. Specially designed rabbit restraining harnesses were fitted to each rabbit at the time of dosing and kept in place during the entire dosing period. These harnesses prevented excessive movement of the rabbits and prevented the rabbit from chewing on the taped area. The harnesses did, however, allow the rabbit access to food and water during the dosing period.

All doses were kept in contact with the rabbit's skin for 24 hours. After 24 hours, the tape, plastic wrap, gauze, and harness were removed. The rabbits were maintained in individual cages postexposure and observed for mortality or other signs of toxicity during the 14 days immediately following exposure.

The animals were observed frequently on the day of dosing and twice daily thereafter. Visible signs of toxicity were recorded. Body weights were obtained at the time of dosing and on days 1, 2, 4, 7, 10, and 14 posttreatment.

Acute Inhalation Toxicity - Concentrated vapors of the test materials were generated in a gas washing bottle equipped with a fritted disk. Dried air was blown at a known rate through the bottle containing a measured amount of test material at a known rate. The resulting air-vapor mixture was conducted to a 60 liter plastic chamber containing 5 male and 5 female rats. Analytical concentrations were not measured; however, estimated concentrations were calculated by material balance.

The animals were exposed for 6 hours and observed frequently during the first day. They were examined twice daily for visible signs of toxicity during the 14 days observation period and weighed on days 1, 4, 7, 10 and 14.

Eye Irritation - One-tenth milliliter of the undiluted test materials was applied to one eye of each of nine albino rabbits. The opposite eye was untreated and served as a control. The eyes of the test animals were examined with fluorescein stain prior to use to ensure absence of lesions or injury. The treated eye of six rabbits remained unwashed while the remaining three rabbits received test material and then had the treated eye

flushed for one minute with lukewarm water starting no sooner than 20-30 seconds after instillation. Examination for gross signs of eye irritation were made at 24, 48, and 72 hours post-application. Scoring of irritative effects was according to the method of Draize (1959) in which corneal, iris, and conjunctival effects are scored separately.

Skin Irritation - A patch test method was utilized to determine the degree of primary skin irritation of intact and abraded skin of albino rabbits.

Six rabbits were clipped of all possible hair on the back and flanks 24 hours prior to exposure to allow for recovery of the skin from any abrasion resulting from the clipping. One of the two test areas on the back was abraded through the stratum corneum, but not sufficiently deep to disturb the dermis or to produce bleeding. This was made in a square pattern with a hypodermic needle used to make the incisions.

Undiluted test materials were applied in the amount of 0.5 ml to the designated patch areas and covered by a one-inch square of surgical gauze two single layers thick. The gauze patches were held in place with strips of Elastoplast® tape. The entire area was covered with polyethylene plastic wrap and secured with more Elastoplast® tape. These patches remained in place on the rabbits for 24 hours. During that time, the rabbits were fitted with leather restraining collars. After 24 hours the wrap and patches were carefully removed and the test areas were evaluated for irritation using the Draize (1959) table as a reference standard. Readings were also made at 72 hours (48 hours after the first reading). If injury occurred, scoring was continued thrice weekly until injury subsided.

Skin Sensitization

A modification of the test described by Maguire (1973) was used. In this test ten individually caged albino guinea pigs, six to eight weeks of age, were used for each material. The materials were tested for primary irritation on three guinea pigs by application to the clipped flank. Observations were made after 24 hours for signs of irritation. If the test material was irritating to the guinea pig skin, dilutions in liquid petrolatum were made to determine the maximum nonirritating concentration.

The test materials were applied at their maximum nonirritating concentration. An area on the back of each animal directly above the forelegs was clipped with electric clippers and chemically depilated with a commercial product on the morning of the first insult exposure. At each application, 0.1 g of the test material was applied to this area on a 1/2 x 1/2 inch cotton gauze square, covered with dental dam, and held in place with adhesive tape. The first insult patch was allowed to remain in place for two days, removed, and a second application of 0.1 gram was made. Two days later, this patch was removed, a total of 0.2 ml of 50% Freund's adjuvant in distilled water per animal was injected intradermally, using 2 or 3 points adjacent to the insult site, then a new patch of 0.1 gram of the test material was applied. On the third day after this application, the patch was removed and a new patch of 0.1 gram of the test material applied. The last patch was removed two days later, and the animals were allowed to rest for two weeks. Each time the insult patches were removed, the condition of the skin at the application site was evaluated and recorded. When the last patch was removed, the toes of the hind feet of each animal were taped to prevent the animal from scratching the irritated area.

After a two-week rest period, both flanks of the animals were clipped and challenged, one side with the test material and the vehicle (petrolatum) on the other flank. The challenge applications were not occluded. The skin responses at these sites were recorded at 24 hours and 48 hours after application. Any animal showing measurable erythema and/or edema at the test solution challenge site was rated as a positive responder.

Acute Delayed Neurotoxicity Testing

Preliminary to the neurotoxicity testing, the acute oral toxicity of the two fluids was determined for chickens. Each hydraulic fluid was administered to nonfasted hens at a concentration of 5 ml/kg body weight. As no deaths occurred, this concentration was used in the acute neurotoxicity test.

The hydraulic fluids, as well as the positive control TOCP, were administered to unfasted hens in an undiluted state. A negative control group received appropriate doses of corn oil. The method follows the proposed guidelines of the Environmental Protection Agency. The following dose regimen was followed:

MLO 82-233:	A group of ten hens received a peroral dose of 5 ml/kg.
MLO 82-585:	A group of ten hens received a peroral dose of 5 ml/kg.
TOCP:	A group of ten hens received a peroral dose of 500 mg/kg.
Corn oil:	A group of ten hens received a peroral dose of 1 ml/kg.

Grading by three observers began on the day following dosing and continued through 21-days after the initial dose.

The following point score was used:

Symptom Free	0 Points
Doubtful of Minor Symptoms	2 Points
Positive Paralytic Symptoms	8 Points
Advanced Paralytic Symptoms	12 Points
Death	16 Points

During observation and grading, the hens were removed from their enclosure and placed on a rubber mat to provide sure footing. Symptoms of test hens noted during the observation period were compared with those seen in the TOCP treated hens. All hens were weighed prior to dosing and once weekly thereafter.

All test and control hens were examined for gross pathology at death. Longitudinal and cross sections of the spinal cord (cervical, lumbar, and thoracic regions) and a section of the sciatic nerve were sampled for histopathology examination.

Because of an equivocal response at 5 ml/kg, the MLO 82-585 neurotoxicity test was repeated increasing the dose level to 7.5 ml/kg and extending the observation period through 30 days, excluding weekends.

Results

Acute Oral, Dermal, and Inhalation Toxicity

Neither hydraulic fluid caused death by the oral, dermal, or inhalation route at the maximum concentration tested as shown in

Tables 83 and 84. Toxic signs were absent and mean body weight gains were normal during the subsequent 14-day observation period.

TABLE 83. ACUTE ORAL, DERMAL, AND INHALATION TOXICITY OF MLO 82-585

Route of Exposure	Species	Sex	Dose	Results No. Dead/No. Dosed
Oral	Rat	M	5.0 ml/kg	0/5
Oral	Rat	F	5.0 ml/kg	0/5
Dermal	Rabbit	M	2.0 ml/kg	0/5
Dermal	Rabbit	F	2.0 ml/kg	0/5
Inhalation	Rat	M	1140 (mg/m^3)[a]	0/5
Inhalation	Rat	F	1140 (mg/m^3)[a]	0/5

[a] Concentration calculated by dividing the amount of test material used by total air volume.

TABLE 84. ACUTE ORAL, DERMAL, AND INHALATION TOXICITY OF MLO 82-233

Route of Exposure	Species	Sex	Dose	Results No. Dead/No. Dosed
Oral	Rat	M	5.0 ml/kg	0/5
Oral	Rat	F	5.0 ml/kg	0/5
Dermal	Rabbit	M	2.0 ml/kg	0/5
Dermal	Rabbit	F	2.0 ml/kg	0/5
Inhalation	Rat	M	1120 mg/m^3[a]	0/5
Inhalation	Rat	F	1120 mg/m^3[a]	0/5

[a] Concentration calculated by dividing the amount of test material used by total air volume.

Eye Irritation

Neither hydraulic fluid caused ocular irritation in the rabbits. No differences were noted when comparing the exposed eyes, washed or unwashed, with the respective control eyes at the scheduled observation periods.

Skin Irritation

The hydraulic fluid MLO 82-233, when applied undiluted to intact and abraded skin, did not produce a primary irritation response. The hydraulic fluid MLO 82-585 was found to be a moderate, reversible primary skin irritant (Table 85). The 72-hour primary irritation index was calculated to be 2.1 which would classify this fluid as a moderate skin irritant. Five days following treatment, no erythema or edema was present. There was, however, minor eschar noted in all six rabbits.

TABLE 85. PRIMARY SKIN IRRITATION OF MLO 82-585

	Mean Skin Reaction Scores					
Time Post	Erythema[a]		Edema[a]		Necrosis[b]	
Dose (Hr)	Intact	Abraded	Intact	Abraded	Intact	Abraded
24	1.2	1.5	0.5	1.7	0	0
72	1.7	1.7	0	0.2	c	c
Day 5	0	0	0	0	c	c
Primary irritation index 2.1[d]						

[a] Erythema and edema - scored on a scale of 0 - 4 with 4 being the most severe.
[b] Necrosis - scored 0, 5, 10, 15 with 15 as the largest area of necrosis.
[c] Minor eschar formation.
[d] Total reaction score (erythema and edema). (Number of animals x Number of observations x Number of test sites).

Skin Sensitization

In the preliminary screening tests, a neat sample of MLO 82-233 and a 1% solution of MLO 82-585 in liquid petrolatum were found to be nonirritating. The results of the sensitization

testing are presented in Table 86. These data indicate that neither of the hydraulic fluids is a potential skin sensitizer.

TABLE 86. SKIN SENSITIZATION POTENTIAL OF MLO 83-233 AND MLO 82-585

Test Material	Conc. %	% Demonstrating Positive Response Post Challenge			
		Control Side		Treatment Side	
		24 hr	48 hr	24 hr	48 hr
MLO 82-585	1.0	0	0	0	0
MLO 82-233	100	0	0	0	0

Acute Delayed Neurotoxicity (21 Days; 5 mg/kg Dose Level)

Mean body weights of the test hens corresponded to those in the corn oil control group. Positive neurotoxic symptoms were observed in all hens receiving TOCP. A TOCP dosed hen was sacrificed after 17 days due to paralysis and inability to obtain food and water. The corn oil control group resulted in all negative or doubtful scores as did the hens that received MLO 82-585.

Nine of the ten hens receiving MLO 82-233 showed negative or doubtful signs through the 21-day period. One hen appeared normal through 19 days; however, when dropped into the observation area on the twentieth day it refused to walk and appeared paralyzed. On the following day the hen was able to stand but showed definite signs of leg weakness. It was not possible to determine if the apparent partial paralysis was chemically caused or a result of the hen landing poorly in the observation enclosure. X-rays failed to show any bone injury and gross pathology was essentially normal.

The delayed neurotoxicity study of MLO 82-233 was repeated using a second group of hens with the modifications described in the methods section. There was no statistically significant difference in mean body weights between the groups at any weighing period. However, a slight decline in mean body weight of the TOCP positive control group of hens was seen at the 14-day weighing period which corresponds with the time that neurotoxic effects began. The low mean body weight of TOCP treated hens continued through the remainder of the 30-day observation period. The MLO 82-233 hens showed a slight decline in mean body weight at the 21-day weighing but recovered by 28 days.

Neurotoxic symptoms were observed in all hens that received TOCP. Two TOCP dosed hens were killed after 24 days on test due to paralysis and inability to obtain food and water. The corn oil treated control group all had negative or doubtful scores.

Nine of the ten hens receiving MLO 82-233 showed negative or doubtful signs through the 30-day period. One hen appeared normal through 11 days; however, on the twelfth day (not a scoring day) the animal technician noted that the hen displayed poor coordination and loss of balance. By the thirteenth day the hen was prostrate and was killed on day fifteen due to its moribund condition.

Conclusions

MLO 82-233 and MLO 82-585 caused no mortality when administered orally or dermally. Neither material caused mortality among male and female rats exposed to saturated vapor concentrations for single six-hour periods and neither material was found to be an eye irritant. MLO 82-585 was determined to be a moderate skin irritant while neither hydraulic fluid demonstrated skin sensitization potential.

The results of the histopathology examination of chicken nerve tissue have not yet been received. Unless the results show definite nerve effects it can be assumed that the response seen in one of ten hens in each of the tests was a toxic response to the hydraulic fluid and that neither of the fluids is a delayed neurotoxin.

SECTION III

FACILITIES

URINARY METABOLITES OF DIMETHYL METHYLPHOSPHONATE

As part of the investigation into the toxicity of dimethyl methylphosphonate (DMMP), experiments were conducted to identify metabolites of this compound in rat urine. Analyses were performed on urine samples from exposed and control rat populations. Intraperitoneal (IP) and inhalation exposures were carried out in order to determine if there were differences in metabolites due to route of administration. Males and females were segregated for urine collection in order to ascertain if

there were metabolite differences attributable to sex. The DMMP employed in these studies was the same material used in the 90-day inhalation toxicity study.

Ten male and ten female Fischer 344 rats received IP injections of neat DMMP at a dose level of 2.0 ml/kg. The dosed rats were placed in metabolism cages for overnight urine collection with food and water provided ad lib. On the following day the urine was pooled by sex and transferred to 4 oz plastic bags (Whirl-pak®) and placed in freezer storage at -80°C. Control urine was collected from untreated male and female rats by the same regime.

Urine was obtained from Fischer 344 rats used in a two-week, range-finding inhalation exposure to DMMP vapor. The animals received a continuous, 14-day exposure to DMMP at a level of 478 ppm. At the termination of this exposure, the nine male and eight female rats that survived were immediately placed in metabolism cages for overnight urine collection. Control samples of urine were obtained from ten male and ten female rats from a sham-exposed population. Collection and storage procedures were identical to those for the urine collected from the IP treated rats as detailed above.

Urine constituents were analyzed by direct injection of untreated urine into the GC/MS and by GC/MS analysis after continuous extraction of urine with ethyl ether as developed for identification of hydrocarbon fuel metabolites (MacEwen and Vernot, 1982). Urine was also extracted after hydrolysis of glucuronides and sulfates to detect conjugated metabolites.

The only compounds detected in the urine of exposed rats different from those in controls were DMMP (with some impurities in the starting material) and γ-butyrolactone. DMMP was present in chromatograms of the serial ether extracts of exposed urine prepared by all three methods. The presence of DMMP in the exposed urine extracts after hydrolysis of conjugates was probably an artifact resulting from the inability to extract all the DMMP from the aqueous urine residue initially, rather than evidence of conjugated DMMP in the urine samples. The test agent impurity, trimethyl phosphate, was found in chromatograms of exposed animal urine extracts which displayed large abundances of DMMP.

Gamma-butyrolactone was identified in extracts of untreated urine and β-glucuronidase treated urine of both male and female rats receiving inhalation and IP exposures to DMMP. No evidence of the lactone was found in the control urine extracts or in DMMP

itself. The γ-butyrolactone peak was small, eluting at about 24.5 minutes. The material was initially found in total ion chromatograms of extracts of IP and inhalation exposed females. Figure 41 is a typical chromatogram. Single ion chromatograms of extracts of urine from exposed male rats demonstrated the presence of γ-butyrolactone in these samples as well.

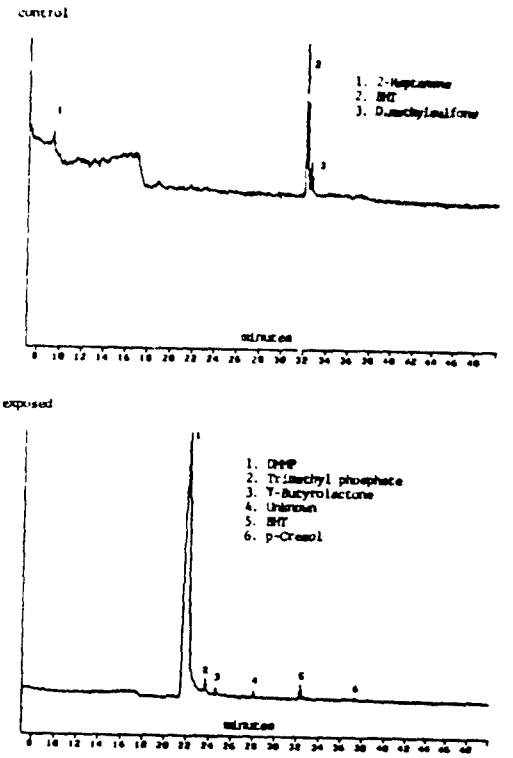


Figure 41. Total ion chromatograms of untreated urine samples from female rats IP dosed with DMMP and contols.

It was uncertain whether the material in the urine was γ-butyrolactone or γ-hydroxybutyric acid which lactonized during analysis, e.g. on injection into the G.C. In order to determine whether γ-hydroxybutyric acid lactonizes in solution spontaneously or on analysis, 0.5 M aqueous solutions of the acid and lactone were prepared. The acid was produced by dissolving the sodium salt in an equimolar solution of hydrochloric acid. Both solutions were then extracted once with an equal volume of benzene and GC/MS analyses performed on the aqueous and benzene portions. In all cases, the compound found was the lactone. However, the benzene/water distribution ratios were different for the two starting materials as shown in Table 87.

TABLE 87. DISTRIBUTION RATIOS OF γ-BUTYROLACTONE AND γ-HYDROXYBUTYRIC ACID

Compound	Peak Area Aqueous Fraction	Peak Area Benzene Fraction	Ratio C_6H_6/H_2O
γ-butyrolactone	885,858	474,276	0.535
γ-hydroxybutyric acid	845,587	9938	0.012

The significant difference in the distribution ratios is presumptive evidence of the integrity of the acid prior to injection. Yet the compound in the aqueous γ-hydroxybutyric acid solution was identified by computer matching as the lactone. Lactonization, therefore, must have occurred during analysis and not in solution.

This investigation has not established which species, γ-butyrolactone, γ-hydroxybutyric acid, or both, is present in the urine of rats exposed to DMMP, but the presence of at least one of the pair has been demonstrated.

INTRODUCTION AND ANALYSIS OF EDMP IN EXPOSURE CHAMBERS

In order to carry out the inhalation exposures to EDMP, methods of introduction and analysis were developed to meet the following objectives:

1. Delivery of the desired concentrations of EDMP with a respirable aerosol particle size distribution to exposure chambers.

2. Determination of the particle size distribution of the aerosols.

3. Analysis of the breakdown products of EDMP in the chambers.

4. Continuous analysis of chamber concentrations.

Each of these objectives was attained in the methods and systems developed.

EDMP Aerosol Introduction System

Figure 42 is a schematic of the system that was used to generate EDMP aerosol into the exposure chamber. The aerosolizer itself was the Solo Sphere® Nebulizer. This nebulizer was used for all exposures of 500 mg/m^3 and above. Above 2000 mg/m^3, two Solo Sphere® nebulizers were used. In that case, all equipment seen in Figure 42 was duplicated except for the house air regulator and the reservoir.

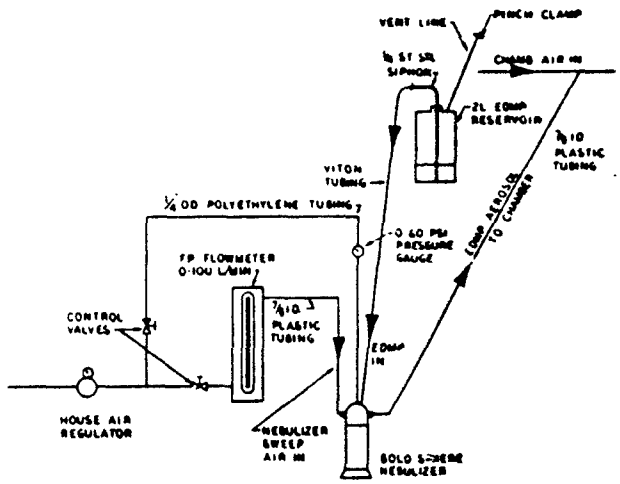


Figure 42. EDMP introduction system.

Dried compressed air was used to operate the system. A needle valve and a gauge were used to control the air pressure between 0-60 psi. The same supply of compressed air was used to sweep the aerosol out of the nebulizer. This was adjusted using a Fischer & Porter 0-100 l/min. flow meter and a needle valve. Flexible plastic tubing was used to supply this sweep air to the nebulizer and to carry the aerosol from the nebulizer outlet port to the chamber main air inlet. EDMP from a 2 L polyethylene bottle reservoir was siphoned into the nebulizer, when needed, through viton tubing.

For chamber concentrations below 500 mg/m^3, a Collison® 3-jet nebulizer was used. The nebulizer was placed inside a 250 ml 3-neck round bottom flask. One neck was used for an aerosol outlet. Attachment to the chamber air input was made using the same plastic tubing as for the Solo-Sphere nebulizer®. A syringe pump

equipped with a 100 ml glass syringe was used to supply EDMP to the Collison® nebulizer. The EDMP was pumped into the flask using 1/16" OD stainless steel tubing.

Chamber concentration was controlled mainly by adjusting the nebulizer air pressure. Chamber air flow rate was also used to control concentration. Depending on target concentration, the air pressure used varied between 3 and 50 psi for one or both nebulizers. Chamber air flow was varied between 10 and 30 CFM. It was found advantageous to use a constant sweep air flow of between 40 and 50 L/min. Increasing or decreasing sweep air flow seemed to have a minimal effect on chamber concentration.

Operation of the Collison® system was similar to the high concentration system. The syringe pump was used to maintain the EDMP level so that the bottom of the nebulizer was immersed. System startup merely involved turning on the air supply pressure which varied from 5 to 60 psi depending on concentration.

Aerosol Characterization

The purpose of this analysis was to characterize the EDMP aerosol introduced into the exposure chamber by determining the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (σg) of the aerosol particles. A secondary purpose was to determine the total aerosol mass introduced into the chamber during each exposure.

The need to generate high concentrations of aerosol in the chamber made the use of automatic aerosol analytical equipment impractical since the Royco particle counter generally can not be used for an aerosol concentration over 5 to 10 mg/m^3. The Mark II Andersen Cascade Impactor which contains eight stages and operates with a constant sample flow of 1 CFM was selected for this application. Table 88 shows the particle diameter range of each stage.

One impactor sample was drawn from the chamber for each six hour exposure. The sample was taken when chamber concentration reached stability. This was usually 1 - 2 hours after exposure start up. Impactor exhaust was directed into a hood. Sample time varied with chamber concentration. With a chamber concentration around 4000 mg/m^3 a 1-minute sample time was used; for a 50 mg/m^3 chamber concentration, a 1-hour sample time was used. A sufficient sample had to be present to be able to measure the amount on each stage, but excessive sample caused aerosol entrainment and error in analysis.

TABLE 88. AEROSOL SIZE RANGES FOR EACH IMPACTOR STAGE

Stage No.	Size Range (Micrometers)
0	9.0 and above
1	5.8 - 9.8
2	4.7 - 5.8
3	3.3 - 4.7
4	2.1 - 3.3
5	1.1 - 2.1
6	0.7 - 1.1
7	0.4 - 0.7
Filter	0 - 0.4

Analysis of impactor samples involved weighing the aerosol collected on each stage. Glass fiber filters were used on each stage as collection media and as a final filter. A computer program was written to calculate MMAD and σg upon entry of the aerosol weights on each stage.

Qualitative Chamber Analysis

In order to determine the amount of EDMP decomposition and to characterize the resulting breakdown products, gas chromatographic analysis was performed during chamber exposures. Previous work had investigated the stability of EDMP when introduced into a chamber as an aerosol (MacEwen and Vernot, 1983) and showed that the stability of EDMP depends primarily on the humidity of the chamber air.

Figure 43 is a schematic of the system used to take GC samples from the chamber. A midget impinger containing 20 ml of absolute diethyl ether was positioned inside the chamber. Chamber air was then pulled through the impinger using a vacuum pump. The EDMP and its breakdown products were trapped in the ether. Ten liters of chamber air were sampled. A wet test meter was used to measure total sample volume and a flowmeter to maintain a nominal sample flow rate of 2.5 liters/min.

Five samples were normally taken during each 6-hour chamber exposure. During sampling, approximately 50 to 70% of the ether evaporated so the ether volume was readjusted to 20 ml after sampling. For low chamber concentrations, ether volume was not adjusted, or in very low chamber concentrations, additional ether was evaporated to further concentrate the sample.

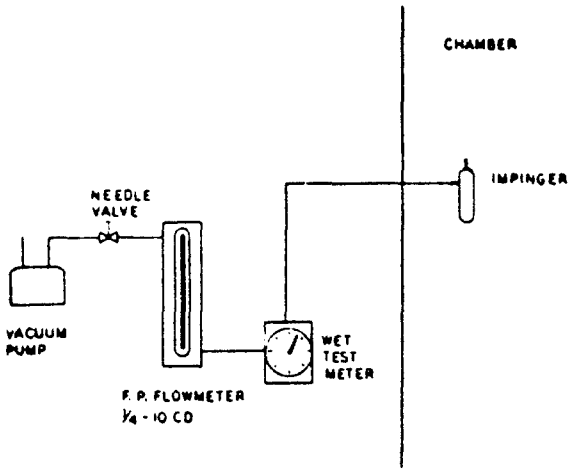


Figure 43. EDMP air sampling system.

Continuous Quantitative Analysis of Chamber Concentration

Previous experiments had shown that the material in an exposure chamber after EDMP introduction would consist of varying proportions of aerosol and vapor and would be a multicomponent mixture containing EDMP and its breakdown products. Absorption into a flowing liquid stream would collect both aerosol and vapors, and infrared analysis was capable of giving correct concentration values under different degrees of decomposition. A choice of 4-methylcyclohexanol (MCOH) as absorbing liquid in the analysis was made because EDMP is soluble in MCOH and because of its low volatility. A zinc selenide (ZnSe) cell was selected because it is transparent to infrared radiation throughout the wavelength range accessible to the Miran IA and is chemically resistant to high water concentrations. A wavelength of 11.6 μm was used because MCOH has a low absorbance here and EDMP has good absorbance. Figure 44 is a recording of the absorbance of a 1.28% solution v/v of EDMP being pumped through the ZnSe cell in the Miran IA for a period of about 1-1/2 hours. Sensitivity of the method is acceptable and stability is good over the analysis period. Figure 45 is a similar recording of the same concentration of EDMP which has been 50% hydrolyzed by the addition of water. Comparison of the mean absorbance of the unhydrolyzed sample, 0.53, with that of the hydrolyzed sample, 0.54, shows that absorbance has been unaffected by hydrolysis and that this measurement provides a reliable estimate of chamber concentration calculated as EDMP even if decomposition has occurred. Figure 46

is a schematic of the analytical system used. The glass absorber tower was positioned in the center of the chamber approximately one foot above the animal cages. Sample air was pulled into the top of the tower at 2.5 L/min. A flowmeter used to maintain a constant flow rate was calibrated by connecting a wet test meter to the inlet of the absorber tower and adjusting the flow rate to 2.5 ml/min.

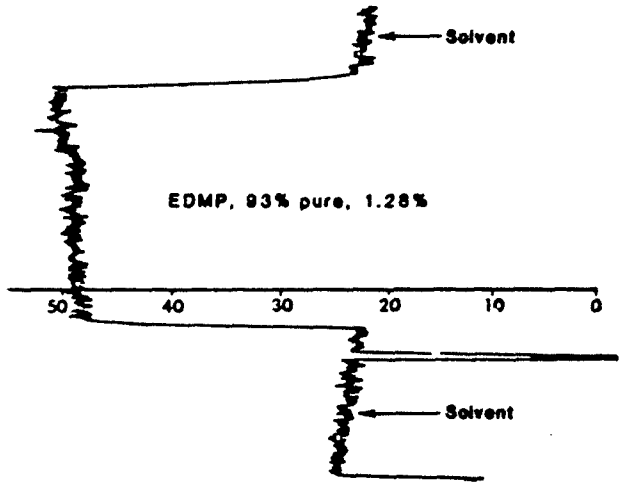


Figure 44. IR analyzer readout of 275 microliters EDMP (93% pure) dissolved in 50 ml of methylcyclohexanol.

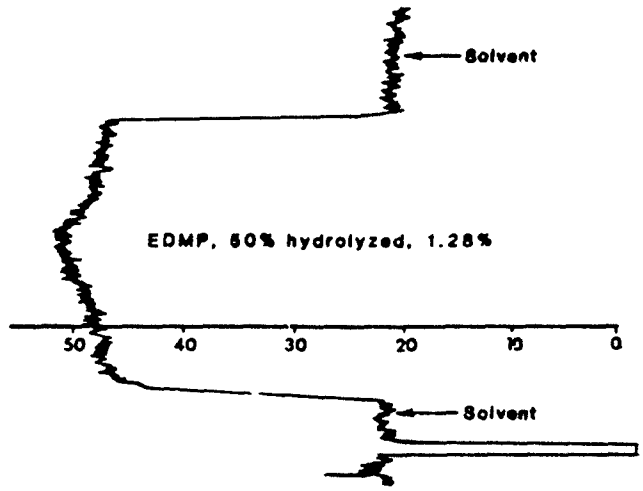


Figure 45. IR analyzer response to 275 microliters of EDMP (50% pure) in 50 ml methylcyclohexanol.

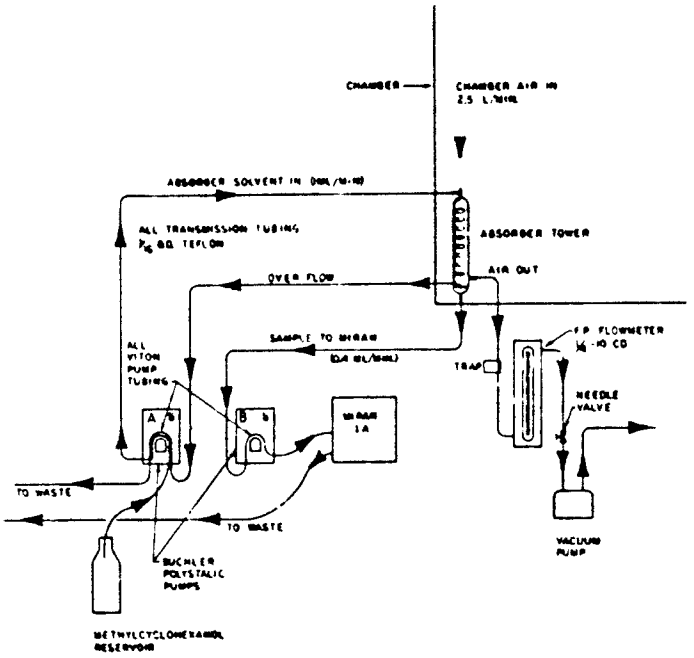


Figure 46. Schematic drawing of the sampling and analysis system for EDMP.

Solution standards equivalent to the chamber concentrations were made up and pumped through the analytical system for 50 minutes under operational conditions. Figure 47 is the calibration curve obtained. The line was generated by computer using a least squares linear regression program. This also calculated a 95% confidence interval for 4000 mg/m^3 to be ± 4.8%.

Analytical calibration below 500 mg/m^3 required modifications to the system. Sensitivity of the Miran was increased, at first, by changing the absorbance setting from 0.1 to 0.025. This allowed chamber exposures to be run down to a level of 125 mg/m^3. For chamber exposures to 50 mg/m^3, the MCOH flow rate was decreased to 0.5 ml/min. By halving solvent flow rate, the Miran response was doubled. Confidence intervals for the lower range calibration curves were also extended; however, this occurred because of the greater noise level and instability of the Miran response when operated at the higher sensitivity levels. At the highest sensitivity level, 50 mg/m^3 was approaching the lower limit of detection.

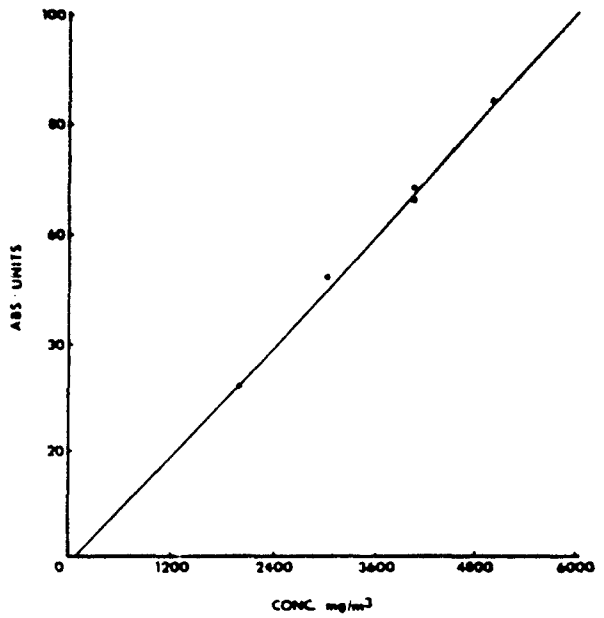


Figure 47. IR Absorption curve for calibration of EDMP analysis.

CHEMICAL ANALYSIS OF THREE TRIARYLPHOSPHATE COMPOUNDS USED IN SMOKE GENERATORS

The Toxicology Detachment of the Naval Medical Research Institute (NMRI/TD) requested that the Toxic Hazards Research Unit (THRU) analyze the phenolic constituents of the following materials qualitatively and quantitatively:

NMRI/TD#	Trade Name/Vendor
2583-1	Chem Chex 200/Tifa. Ltd.
2583-2	Kronitex 100/FMC Corp.
2583-3	Residual Fluid

These materials are triarylphosphates whose chemical and toxicological properties are dependent on the nature of the phenols esterifying the phosphoric acid.

In response to the request of NMRI/TD, the THRU analyzed hydrolysates of the three compounds for o-cresol content, essentially by the method given in military specification number MIL-H-19457C(SH). A five-gram sample of each material was saponified

overnight in a Parr bomb containing aqueous caustic solution. After neutralization, the phenolic fraction was extracted with diethyl ether. The ether solution was injected into a GC and the o-cresol quantity determined after standardization using known mixtures of o-cresol in diethyl ether. Gas chromatographic/mass spectrometric (GC/MS) analysis was then performed on the hydrolysates to give a positive identification of the components.

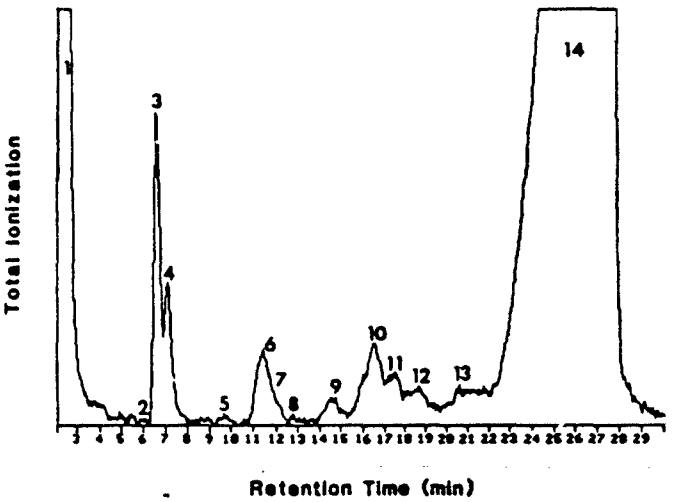
The results of GC analysis of o-cresol in the triarylphosphate fluids are presented in Table 89.

TABLE 89. O-CRESOL CONCENTRATIONS OF THREE TRIARYLPHOSPHATE COMPOUNDS

NMRI/TD Sample	o-Cresol Concentration[a] ± SD
2583-1	0.0090 ± 0.0012
2583-2	0.0480 ± 0.0020
2583-3	0.0092 ± 0.0013

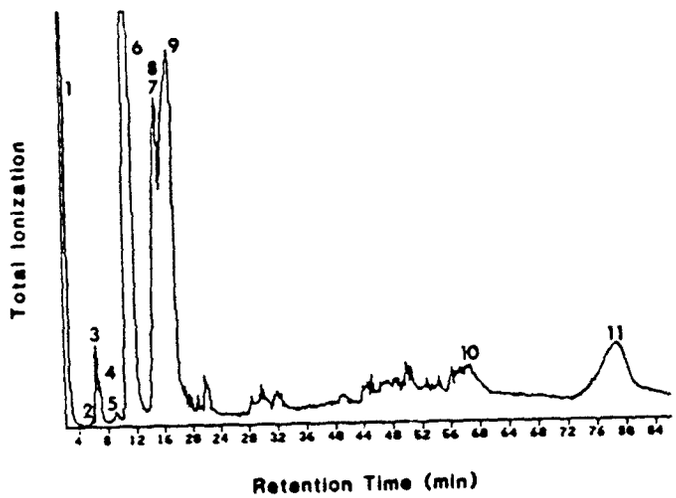
[a] Weight percent of the original sample.

The GC/MS total ion chromatograms, identifications, retention times, and area percents are shown in Figures 48, 49, and 50, respectively. The area percent value is a percent concentration of the total extracted phenolics. Most of the components were identified but the mass spectra of the isomers of some compounds are so similar that they could not be distinguished. The xylenols, isopropylphenols and diisopropylphenols are listed, but the isomers are not specified for this reason. Samples #2583-1 and #2583-3 give almost identical GC curves. About 90% of these materials consist of phenol and p-t-butylphenol. Sample #2583-2 is quite different and consists primarily of mono and disubstituted isopropylphenols and phenol. Figure 51 is a total ion chromatogram of phenols in Fyrquel 220 hydraulic fluid reported in a letter report to NMRI/TD dated 9 September 1982. The similarity between the GC curves of Fyrquel 220 and the NMRI/TD samples #2583-1 and #2583-3 is evidence that these samples and Fyrquel 220 contain the same chemical constituents and in approximately the same proportions.



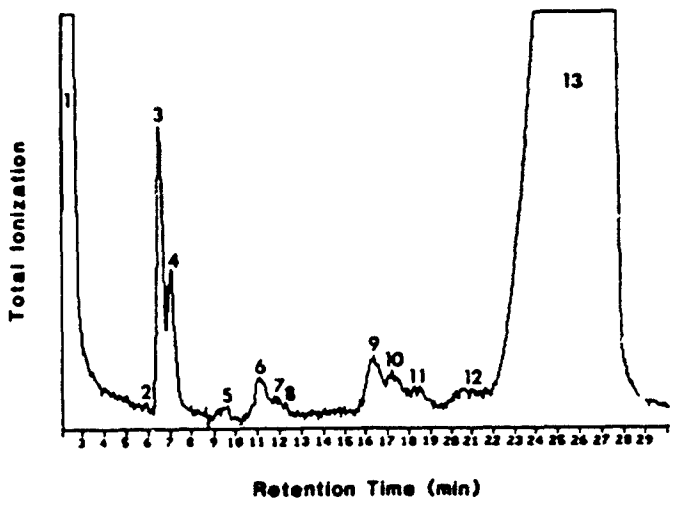
Peak #	Retention Time (min)	Peak Identification	Area %
1	2.2	Phenol	33.1
2	6.0	o-Methylphenol	0.03
3	6.6	m-Methylphenol	2.2
4	7.5	p-Methylphenol	1.1
5	9.8	o-Ethylphenol	0.1
6	11.1	m-Ethylphenol	0.5
7	11.6	Isopropylphenol	0.5
8	12.2	----------	0.3
9	14.6	Isopropylphenol	0.3
10	16.2	o-t-Butylphenol	1.4
11	17.5	Xylenol	0.6
12	18.7	Xylenol	0.5
13	21.1	m-t-Butylphenol	0.3
14	25.8	p-t-Butylphenol	59.1

Figure 48. Total ion chromatogram of triarylphosphate compound NMRI/TD 2583-1.



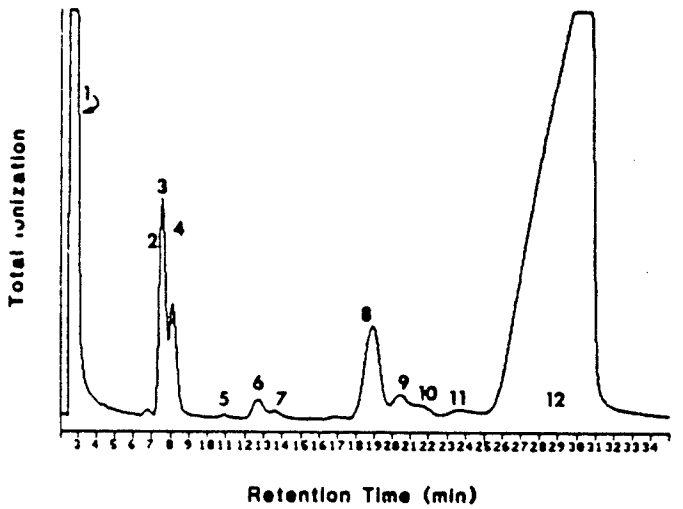
Peak #	Retention Time (min)	Peak Identification	Area %
1	2.2	Phenol	27.9
2	5.9	o-Methylphenol	0.1
3	6.5	m-Methylphenol	1.0
4	7.1	p-Methylphenol	0.5
5	9.2	o-Ethylphenol	0.1
6	11.8	Isopropylphenol	22.4
7	14.9	Isopropylphenol	13.2
8	16.3	Xylenol	9.2
9	18.0	Isopropylphenol	14.3
10	58.2	Diisopropylphenol	2.9
11	78.6	Diisopropylphenol	8.4

Figure 49. Total ion chromatogram of triarylphosphate compound NMRI/TD 2583-2.



Peak #	Time (min)	Identification	Area %
1	2.2	Phenol	31.3
2	5.9	o-Methylphenol	0.04
3	6.6	m-Methylphenol	2.1
4	7.5	p-Methylphenol	1.2
5	9.4	o-Ethylphenol	0.2
6	10.9	m-Ethylphenol	0.5
7	11.6	----------	0.2
8	12.2	p-Ethylphenol	0.1
9	16.3	o-t-Butylphenol	0.9
10	17.5	Xylenol	0.6
11	18.5	Xylenol	0.3
12	20.8	m-t-Butylphenol	0.3
13	25.7	p-t-Butylphenol	62.1

Figure 50. Total ion chromatogram of triarylphosphate compound NMRI/TD 2583-3.



Peak #	Time (min)	Identification	Area %
1	4.3	Phenol	24.5
2	6.8	o-Methylphenol	0.1
3	7.6	m-Methylphenol	5.3
4	8.6	p-Methylphenol	1.1
5	11.0	o-Ethylphenol	0.1
6	12.7	m-Ethylphenol	0.6
7	14.0	p-Ethylphenol	0.2
8	18.8	o-t-Butylphenol	4.1
9	20.6	Xylenol	1.1
10	22.0	Xylenol	0.3
11	23.7	m-t-Butylphenol	0.2
12	29.3	p-t-Butylphenol	62.5

Figure 51. Total ion chromatogram of Fyrquel 220.

ALTERATIONS IN ALVEOLAR CLEARANCE AFTER 4-IPOMEANOL INDUCED NECROSIS OF CLARA AND CILIATED CELLS IN THE TERMINAL BRONCHIOLE OF THE RAT

Introduction

In a current model, pulmonary clearance is dependent on the continuous, ciliary propulsion of a double layer of supraepithelial fluid (Plopper et al., 1973; Stephens et al., 1972). The upper, viscous gel or mucus layer contains entrapped pollutants and is propelled by the claw-like tips of underlying cilia which

are beating in the subjacent low-viscosity periciliary fluid. Although the source of the mucus is thought to be mucous glands and goblet cells (Kilburn, 1968), the source of the periciliary fluid is uncertain. The periciliary fluid in the terminal bronchioles might be secreted by either the Type II alveolar cells (Kilburn, 1968), the microcilia of ciliated cells (Reid et al., 1983; Spicer et al., 1983) or the nonciliated bronchial epithelial (Clara) cells (Kilburn, 1968; Ebert et al., 1976; Gil and Weibel, 1969).

4-Ipomeanol (4-IPO), a naturally occurring chemical derived from moldy sweet potatoes, is converted by a cytochrome P450-dependent pathway in the Clara cells to a highly reactive metabolite which is, at low doses, selectively toxic to the Clara cells in the terminal bronchiolar epithelium (Boyd, 1980). At higher doses both ciliated and nonciliated cells are destroyed (Boyd, 1980). Therefore, 4-IPO induced necrosis of these cells provides a model to test the functional role of the Clara cell in alveolar clearance and evaluate the effect of extensive necrosis of the terminal bronchiole on alveolar clearance. Since inhalation of lung irritants such as nitrogen dioxide and ozone also produces bronchiolar lesions, the 4-IPO system may serve as a paradigm for their action as well.

Rats were given ip doses of 4-IPO and the lung effects evaluated by light microscopy of sections sampled over the postexposure period until recovery. The concurrent clearance rates were evaluated by following the decreases in thoracic radioactivity after instillation of ^{51}Cr-labeled microspheres (MacEwen and Vernot, 1983).

Methods

Eighteen hours prior to the 4-IPO ip injection, ^{51}Cr- labeled polystyrene latex microspheres (PSL) were instilled via the trachea into the lungs of 60 rats [20 each in the control (C) group; low dose (LD) group, 10 mg/kg; and high dose (HD) group, 25 mg/kg]. Serial external thoracic radioactivity measurements were made immediately after instillation, just prior to 4-IPO injection, and then at specific intervals through 40 days post-instillation. For light microscopy evaluations, six rats (two per control and each treatment group) were sacrificed at 1, 3, 7, 11, 15, 20, and 40 days following 4-IPO injection.

Sprague-Dawley male rats (Charles River), 175-255 g were used. Cultures for Mycoplasma pulmonis from rats sacrificed for quality control determinations were all negative. The animals were given food (Purina Formula #5008) and softened water (< 17 ppm calcium carbonate) ad libitum. The animals were caged in individual, wire-bottom cages.

The 4-IPO (1-[3-furyl-4-hydroxy-1-pentonone]) was donated by Dr. Michael R. Boyd (NIH Bethesda, MD). The solution received was analyzed by mass spectrometry (Hewlett-Packard 5985, solid probe), and the spectrum agreed with the 4-IPO spectrum previously reported (Boyd et al., 1973). Suspensions of 4-IPO were prepared in an aqueous solution of 25% PEG 400 immediately prior to ip injection with the three dose groups of 0, 10, and 25 mg/kg 4-IPO.

In order to provide rapid fixation and to maintain the dimensions and configurations of the lung tissue at approximately total lung capacity, the lungs taken for micropathologic examination were fixed by airway perfusion with fixative at a constant perfusion pressure of 20 cm of water. A modified Karnovsky's paraformaldehyde/glutaraldehyde fixative was used with added calcium chloride and cacodylic acid after pH adjustment to 7.2 (Dungworth et al., 1976; Nowell and Tyler, 1971). After fixation, the left lobe was bisected longitudinally and embedded in paraffin blocks for slide preparation.

The clearance ability of the lung was evaluated by measuring the radioactivity of retained radiolabeled microspheres deposited in the lungs. Polystyrene latex microspheres (MMAD = 1.6 μ, geometric standard deviation = 1.3) labeled with ^{51}Cr were used (Applied Polymer Technology, Costa Mesa, CA). After anesthetization with 4% Halothane-oxygen mixture, the rats were intubated using a stainless steel speculum (Nicholson and Kinkead, 1982), and 0.3 ml of the 0.1% suspension of microspheres was instilled. For radioactivity measurements, the rats were placed within an annulus-shaped NaI (T1) detector shielded by lead. The detector was used with a multichannel analyzer (Canberra, Meriden, CT) which integrates counts in the energy region of interest. All measurements were made for a sufficient period of time to obtain 1000 counts.

Residual analysis showed that a two phase curve of form $Y = A_1\exp(-B_1t)+A_2\exp(-B_2t)$ was needed to fit the alveolar radioactivity clearance curve in control rats. Measurement of the alveolar clearance curve began at least 24 hours after particle instillation in order to allow for clearance of particles

deposited on the tracheobronchial tree. Prior to curve fitting, the data were corrected for the decay of the ^{51}Cr. Because of the delay in clearance produced by 4-IPO in the HD rats, the two-phase curve was fit using only the data obtained after clearance began. Comparisons among the treatment groups of intercepts of each phase were made by a one-way analysis of variance followed by Duncan's test. The Kruskal-Wallis method followed by Dunn's nonparametric method of multiple comparisons were used for comparison of the half-life data. An F ratio or chi-square value with $p < 0.05$ was defined as statistically significant.

Toxicity

Probit analysis of preliminary toxicity study data established the two week LD_{50} for ip administered 4-IPO at 21.1 ± 1.9 mg/kg (SD). During the clearance study, 1 of 20 LD rats and 7 of 20 HD rats died, and their data were excluded from the results.

Light Microscopy

One day postinjection the most striking lesions observed in the exposed rats were degeneration and necrosis of the terminal bronchiolar epithelium (Figure 52). Degenerative changes ranged from acute cellular swelling to necrosis. The latter condition was accompanied by exfoliation of both the ciliated and non-ciliated bronchiolar epithelium with exposure of the underlying basement membrane. The severity of these changes was dose-dependent with an average severity grade of mild in the LD group and moderate in the HD group. Minimal, acute cellular swelling of scattered bronchiolar epithelial cells was also noted in the intermediate and more proximal bronchioles, but necrosis and exfoliation were not evident. Acute pulmonary perivasculitis and peribronchiolitis were present in treated rats and absent in the controls. These lesions were generally restricted to the level of the terminal and intermediate bronchioles where minimal to mild perivascular and peribronchiolar infiltrates of mixed inflammatory cells and fibrinous debris were present. In the HD rats, these changes were sometimes associated with minimal to mild, multifocal, acute alveolitis and edema.

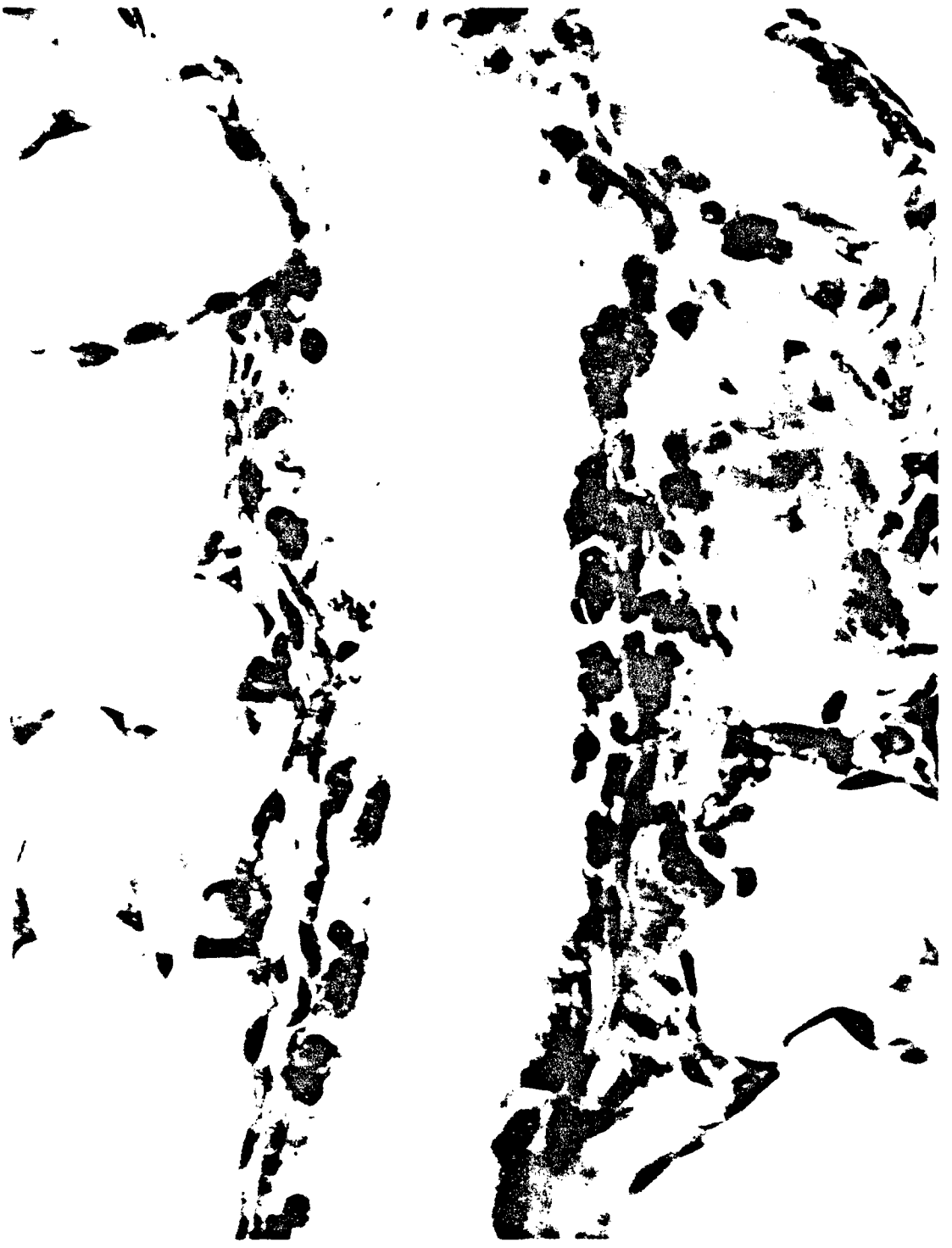


Figure 52. Terminal bronchiole 1-day postinjection with 25 mg/kg of 4-IPO, showing degeneration, necrosis, and exfoliation of ciliated and nonciliated epithelium. (Hematoxylin - eosin stain, original magnification: x 100).

By day 3, the lesions were less severe. Only multifocal, mild degeneration, and necrosis of the terminal bronchiolar epithelium were seen, and only in the HD subjects. There was essentially no microscopic evidence of injury to the more proximal bronchioles, and exfoliated terminal bronchiolar epithelium of the LD rats had been replaced by regenerating low cuboidal epithelium, while repair in the HD rats was limited to a less-differentiated, simple, squamous epithelium (Figure 53). All of the HD rats manifested minimal, acute to subacute perivasculitis and peribronchiolitis. This was not present in the LD rats. No alveolar alterations were noted in experimental rats. A slignt increase in the number of alveolar macrophages, however, was noted either in the bronchioles or alveoli of exposed rats where they were considered to be a normal postnecrotic inflammatory response.

Re-epithelialization of terminal bronchiolar epithelium was almost complete at day 7 (Figure 54). The lungs of one HD rat were morphologically indistinguishable from those of the control animals, while the other HD subject still displayed a few terminal bronchioles with epithelium in the transitional stage between squamous and normal cuboidal epithelium.

On day 10 the terminal bronchioles of both HD subjects were morphologically indistinguishable from the control rats (Figure 55). A slight increased incidence of perivasculitis/peribronchiolitis was present in exposed groups when compared to the controls.

Pulmonary Clearance

Figure 56 shows the decrease with time of thoracic radioactivity expressed as a percent of the initial activity obtained immediately after PSL particle deposition. The shaded area is the 95% confidence zone of the mean control data. Clearance through 1-day postdeposition was considered to represent removal of particles deposited on the tracheobronchial surface while clearance during subsequent days reflected alveolar clearance.

During the early postexposure period when the terminal bronchioles were undergoing necrosis, the HD rats showed a delay for approximately 55 hours in which clearance was almost stopped.

Figure 53. Terminal bronchiole 3 days postinjection with 25 r /kg of 4-IPO, showing single squamous re-epithelialization. (inset). A few clumped, free cells can be seen in bronchiolar lumen. (Hematoxylin - eosin stain, original magnification: x 50; inset, x 100).

Figure 54. Terminal bronchiole 7 days postinjection with 25 mg/kg of 4-IPO, showing low cuboidal re-epithelialization (inset) and occasional cells with cilia (arrows).
(Hematoxylin - eosin stain, original magnification: x 50; inset, x 100).

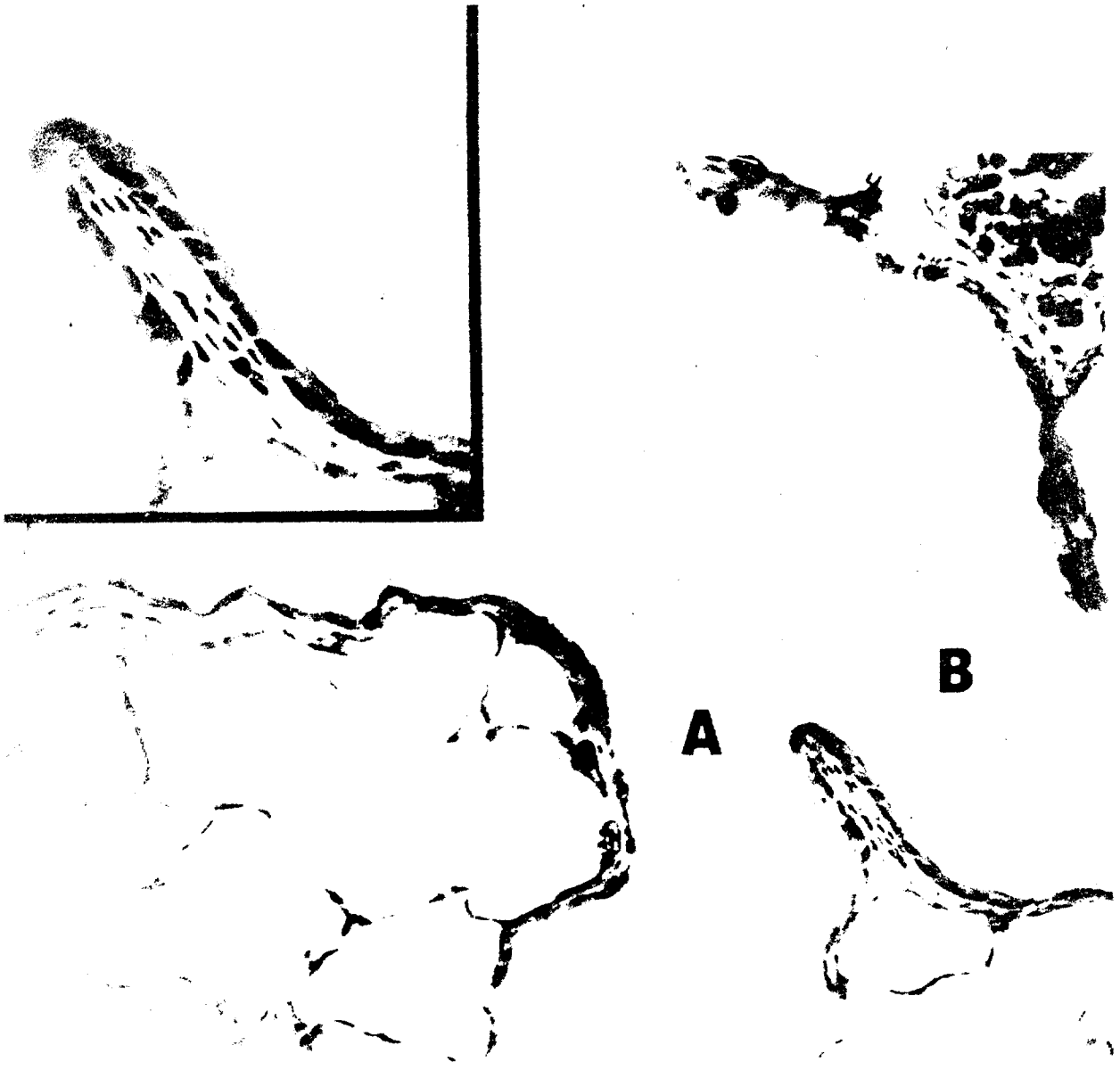


Figure 55. Terminal bronchioles (A and B) 10 days postinjection with 25 mg/kg of 4-IPO, showing bronchioles lined by cuboidal epithelium, many cells with prominent cilia (inset), and essentially indistinguishable from controls. (Hematoxylin - eosin stain, original magnification: x 50; inset, x 100).

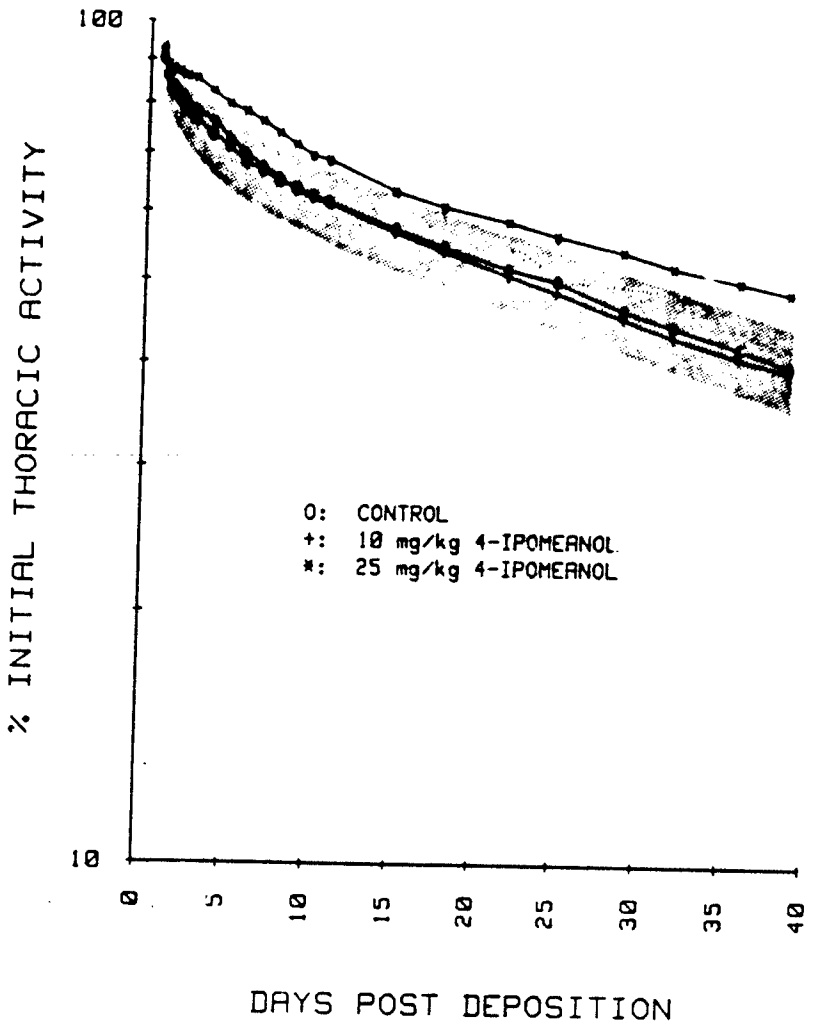


Figure 56. External thoracic radioactivity versus time post radiolabeled particle deposition. The 4-ipomeanol ip injection occurred at 18 hours postdeposition. The shaded area represents 95% confidence limits on mean value of control data.

Subsequently, during phase 1 of the alveolar clearance, both the LD and HD rats showed a significantly reduced clearance rate when compared with controls. Table 90 presents the calculated intercept and half life for each of the two phases fit to the

postdelay data in the alveolar clearance curve. The phase 1 intercept for the HD rats was significantly elevated, but this would be expected due to the delay noted above.

TABLE 90. INTERCEPT AND HALF LIFE VALUES FOR EACH PHASE OF A DOUBLE EXPONENTIAL FUNCTION FIT BY NONLINEAR REGRESSION TO THE POST 4-IPO INDUCED DELAY ALVEOLAR CLEARANCE CURVE

4-IPO Dose	Intercept, %*	Half Life, Days†	Intercept %*	Half Life, Days†	N
Control	20.4 ± 1.3‡	2.5 ± 0.28‡	70.2 ± 3.2‡	51.3 ± 3.2‡	20
10 mg/kg	23.4 ± 1.5‡	3.5 ± 0.25§	68.0 ± 4.8‡	51.2 ± 2.2‡	19
25 mg/kg	31.4 ± 2.8§	5.4 ± 0.66§	68.5 ± 7.1‡	101.1 ± 18.4§	13

x ± S.E.

Groups within each column with a different symbol (‡, §, ¶) are significantly different by (*) One way ANOVA/Duncan, or (†) Kuskal Wallis/Dunn (< 0.05).

During phase 2 of the alveolar clearance curve, on days 10 through 40, only the HD rats had significantly decreased clearance. The linearity of the HD rats clearance curve during phase 2 indicates no reversal or return to the normal clearance rate. The phase 2 intercepts were not significantly different.

Discussion

The terminal bronchiole is the primary target site for 4-IPO toxicity in the rat lung. Morphologically, one day after 4-IPO injection, the lungs demonstrated acute, dose-related injury. In contrast, animals examined at three days postinjection showed a mixture of less severe degenerative changes and the onset of repair. This trend was confirmed at day 10 by the normal appearance of pulmonary target sites in all dosed animals. Fourteen days after 4-IPO injection, all exposure-related microscopic lesions had been repaired so that there were no morphologic differences among exposure and control groups. The morphologic changes reported here agree with those recently published (Boyd, 1980; Doster et al., 1983; Haschek et al., 1984) on the effects of 4-IPO on the lung.

Alveolar Clearance

The microscopically detectable structural alterations present in the acute insult period suggest the possibility of impaired pulmonary clearance. The destruction of the distal portion of the mucociliary escalators might obstruct the removal of particles from the alveoli. The early clearance data for the HD group indicates that this did occur. Clearance was almost completely arrested during the time cellular debris was present and resumed as soon as it was removed.

During the early repair period in the HD rats there were no ciliated cells and none of the proliferating cells resembled mature functioning Clara cells. If the depleted Clara cells or microcilia in the ciliated cells were the source of the periciliary fluid needed for clearance of alveolar material up the mucociliary ladder, then during this time period clearance should be reduced and as the Clara and ciliated cells return to normal, clearance should return to normal. The clearance data for the HD rats did show a significant decrease in clearance shortly after instillation. However, clearance rates were still reduced below control values even when microscopic appearance suggested that tissue repair was complete. Therefore, the decreased clearance does not correlate with morphologic repair of Clara or ciliated cells and appears to be long term. Further studies are currently underway to determine if functional as well as morphologic repair of the Clara or ciliated cells has occurred.

Possible mechanisms for decreased clearances rates include: 1. Hyperplasia during cellular recovery, narrowing the lumens of the bronchioles (Creasia, 1979); 2. Edema resulting from treatment (Sabo et al., 1983); 3. Reduced recruitment of macrophages possibly by suppression of a chemotactic factor. In this study, micropathologic examination demonstrated no hyperplasia during recovery and edema was minimal and resolved even when clearance rates were depressed. Therefore, the first two hypotheses do not seem to be likely causes. Ferin (1982) recently reported that phase 1 alveolar clearance is almost totally mediated by alveolar macrophages.

Adamson and Bowden (1982) have shown that, six hours after exposure to a dust, the supernate of lavaged fluid contains a chemotactic factor(s) which increases alveolar macrophage numbers for days after the exposure. They concluded that this chemotaxin induced recruitment of macrophages both from circulating blood monocytes and by proliferation of interstitial precursors.

Forty-eight hours after the exposure, the lavaged fluid does not contain the chemotactic factor.

The 4-IPO used in our studies may have interfered with production or function of the chemotactic factor during the critical time for macrophage recruitment. We were not able to compare numbers of macrophages in control and exposed lungs because lavage studies were not carried out in these experiments, and perfusion of the airways during fixation removed the macrophages.

The intercepts of phase 1 and phase 2 portions of the clearance curves were the same in all groups tested (when the HD phase 1 intercept was corrected for delay in clearance). This is evidence that particles had been segregated into fast and slow clearance compartments before administration of 4-IPO. A similar result has been reported by Ferin (1982) after the chlorphentermine-induced phospholipidosis of the macrophages was stopped. After the alveolar macrophages recovered, clearance recovered and caught up with the control clearance. In like manner, Creasia (1979) demonstrated that 60 ppm NO_2 exposures produced a delay in clearance, but clearance then accelerated to return to the normal clearance curve.

Certainly the destruction of the terminal bronchiolar epithelium could have interfered with the production or function of this chemotactic factor during the critical time period for macrophage recruitment and interstitial proliferation. However, because we fixed the lungs by airway perfusion, and as a result washed out the macrophages, we have no morphologic data which indicate any change in the number of alveolar macrophages.

If chemotaxin inhibition was the mechanism for the observed decrease in clearance, then this would also support the theory that there is a threshold of particles needed for production of this factor and recruitment of additional macrophages. The factor is apparently there early after the exposure when there are a large number of particles. The factor does not appear to be there, however, after the terminal bronchiole is repaired and a small but additional amount of particles are still there due to the decreased clearance. This threshold effect has been reported by Adamson and Bowden (1981) who demonstrated that high doses of carbon induced increases in macrophages whereas low doses did not.

Particle Distribution

Interestingly, the intercepts in phase 2 were not significantly different between the control and exposed rats. The high dose phase 1 intercept was different from control and LD groups, but that would be expected due to the delay produced by the more extensive necrosis observed in the HD rats. If a double exponential curve accurately models clearance (i.e. no delay), the intercept represents that amount of particles being cleared by whatever mechanism is predominant during that time period. This is a function of both the pattern of deposition and clearance. We know the deposition pattern is not different between the exposed and control rats because they were both dosed using the same technique prior to the 4-IPO treatment, and they had identical external thoracic radioactivity counts immediately after microsphere instillation. Therefore, this implies that the distribution between phase 1 and 2 was not affected by the 4-IPO induced increased particle residence time in the alveoli, which is a somewhat surprising result.

The lack of a shift in distribution of particles cleared during phase 1 or 2 is not without precedent, however. A similar result has been reported by Ferin (1982) after the chlorphentermine-induced phospholipidosis of the macrophages was stopped. After the alveolar macrophages recovered, clearance recovered and caught up with the control clearance. Similar results were also seen by Creasia (1979) where 60 ppm NO_2 exposures produced a delay in clearance, but, afterwards, clearance accelerated to return to normal.

Terminal Bronchiolar Necrosis

One distinguishing difference between the LD group where the delay in clearance was reversible and the HD group where the decrease in clearance was long-term is the degree of insult within the terminal bronchiole. The HD group had virtually complete destruction and replacement of the ciliated and nonciliated cells in the bronchiolar epithelium as compared to only partial destruction in the LD group. Therefore, total necrosis of the terminal bronchiole produced by 4-IPO, and potentially any other terminal bronchiolar toxin, caused a significant delay and decrease in alveolar clearance which did not return to normal even after return of morphologically normal epithelium. Such a decrease would concomitantly increase the alveolar residence time of deposited materials and therefore increase the exposure to these materials and their toxicity.

IMPROVEMENTS TO THOMAS DOME CHAMBER OPERATIONS

Modification of Chamber Input and Exhaust Control Systems

During the last year both older and newer Thomas Dome chamber control systems underwent extensive remodeling to reposition instrumentation, valves, and piping in an orderly fashion.

The newer chambers were modified first. These were easier to alter because of the space provided at each dome. In each case the instrumentation, piping, valves, and air header were neatly divided into the air input and air exhaust sections. By arranging them in this way, malfunctions can be detected easily, and less than one-half hour is required to change out any component. The older chambers presented a problem because instrumentation, valves, and piping assemblies for two chambers had to be constructed on each side in a limited space. To accomplish this, the air input piping was modified and an extension was added to the existing instrument station to separate each dome control system. Since each chamber needed a separate air header for all the pneumatic instrumentation, a staggered arrangement was constructed which fit neatly behind the instrument station. Constructed in this fashion, the older chambers could be easily maintained like the newer ones.

The modifications of the control stations have simplified maintenance and improved operational integrity over the past six months since the change was initiated. These changes, coupled with a manual override system which is still being installed, will give the exposure chamber control operation better reliability during year-long studies.

Contaminant Introduction Modification

In the original configuration for contaminant introduction, the contaminant, contained in an air stream, entered the chamber air duct through 1/2" diameter tubes. The tubing joined the 3" air input pipe, continued to the center, and was bent 90° to extend parallel to and in the direction of air flow. Mounted in this way, with the tubing pointing upward, there was occasional condensation of the contaminant in the tubing which made it difficult to maintain precise concentrations in the chamber.

In order to minimize tubing surface available for condensation, a modification of the introduction spool piece and port was implemented. The 1/2" long radius 90° elbows were removed and replaced with a straight 3/4" tube flared 30° from the top

(Figure 57). This type of construction allows more mixing to occur in the air stream with the contaminant injected at a 90° angle to the flow. These new contaminant spool pieces and piping have been operating successfully for over six months with no indication of condensation in the introduction system.

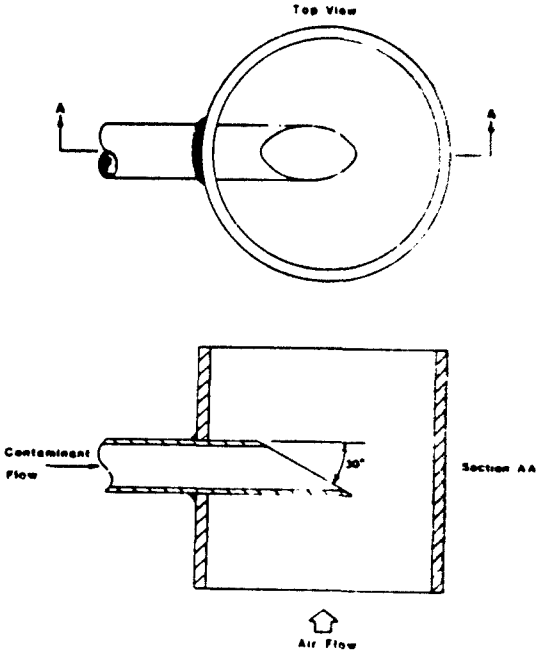


Figure 57. Contaminant input nozzle.

Emergency Air Supply System

The Emergency Air Supply System, as shown in Figure 58, was designed to supply breathing air from a compressed air tank to personnel wearing breathing masks or hoods in the ambient laboratory or within the Thomas Dome chambers. The design is intended to provide measurement of the carbon monoxide concentration of compressor-supplied air and to switch automatically to tank air supply upon compressor failure.

The main air compressor draws its air supply from an area where engine exhaust might raise the carbon monoxide content above the safe breathing level. A Biosystem Series 5000 Monitor was installed to monitor the air before it entered the breathing system and is set to alarm at 50 ppm carbon monoxide. Once the

alarm is activated, chamber safety observers will switch from the main air system to the emergency air system, thus giving personnel wearing breathing equipment a continual safe air supply.

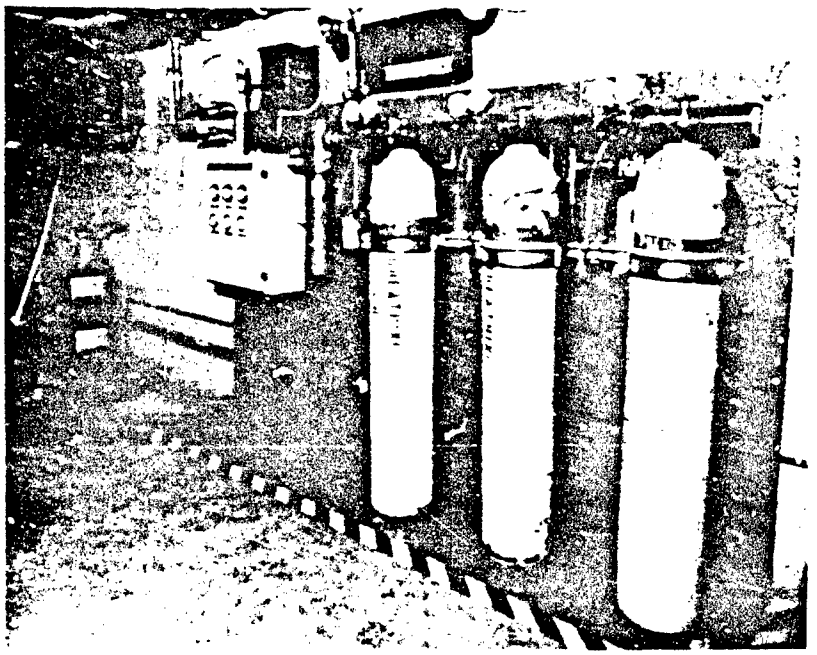


Figure 58. Exposure chamber personnel air supply system.

The Emergency Air Breathing backup system is designed to detect loss of air pressure from the main compressor system and switch to a tank breathing air supply automatically so that dome and laboratory operation will not be interrupted. This switching was accomplished by the use of a pneumatically operated valve that switches tank air into the system once pressure drops below 50 psig. Three 2500 psig breathing quality air tanks were installed into the system to be used in sequence instead of all at once so empty bottles could be replaced if prolonged use of the emergency air system was needed. These bottles are triggered sequentially by a pneumatic valve similar to the one which is activated on compressor failure when tank pressure drops below 50 psig. The output of the 2500 psig tank is regulated to 50 psig for use in the system. The Emergency Breathing Air System provides much needed protection from carbon monoxide contamination and loss of compressor-supplied breathing air.

Vacuum Pump Protection

In September 1983 the Sullair air compressor was shut down during a partial building power failure. This compressor provides air to many systems in Building 79, including the air which controls the diaphragm valves providing cooling water to the Thomas Dome vacuum pumps. When the shutoff occurred, the valves closed and cooling water was cut off from the pumps which continued to run. Two pumps were running at the time and, when casing paint began to smolder because of high heat, chamber technicians noticed the smoke and shut off the pumps. By this time both oil and water seals had been damaged.

This event pointed out the need for a better protection system for the vacuum pumps that would not permit the equipment to operate out of limits to the extent of causing damage. One element of the existing control system, if functioning properly, could have protected the pumps from damage. Each pump had an overtemperature control switch which utilized a fluid-filled bellows system with a remote sensing bulb located in the pump exhaust line. Unfortunately, these switches were not functional during the compressor failure and did not shut off the pumps. In addition to this problem, there was no interlock between the pumps and cooling water, each system being independent.

Given these conditions, the plan for corrective action included the following work to adequately protect the pumps, regardless of the circumstances.

1. Replacement of the overtemperature switch with new switches having a lower sensing range and immersion wells for protection from possibly corrosive contaminants. The lower sensing range makes up for the temperature lag caused by the protective immersion well over the sensing bulb.

2. Replacement of existing pneumatic diaphragm valves that control the cooling water with electric solenoid valves that use the same power source as the vacuum pumps so that the pumps and cooling water valve are using the same power supply and if one loses power both lose power.

3. Installation of water flow sensing switches in each pump to shut it off in case of cooling water loss for any reason. This will also prevent the recurrent problem of pumps being activated without cooling water valves being

opened. The pumps will activate upon depressing the start button with or without water flow. However, if there is no water flow, the pumps will deactivate as soon as the start button is released. If water is flowing the flow switch will close during the time it takes to press the start button, enabling the pumps to remain energized when the start button is released.

In summary, these controls will serve to tie the pump and water supply together so that one cannot operate without the other. The functional overtemperature switch provides a redundancy that will insure the pumps cannot damage themselves even if the flow switch and electrical solenoid valve malfunction.

Vacuum Pump Vibration Isolation

Transmission of vacuum pump vibration to the chamber air exhaust pipe and to the building structure had aggravated the chamber noise situation, giving the impression that more low frequency noise existed than was actually the case.

The primary source of vibration was from the vacuum pump mufflers whose vibration travelled to the dome room partly through the exhaust manifold and piping. To isolate the three mufflers, rubber spool sections were installed downstream of each muffler. Pump mufflers were also suspended from the support frame by spring hangers in an effort to absorb additional vibrational energy.

The three pumps are bolted to one large stand which, in turn, is bolted to a concrete slab floor. To isolate the stand in the most effective way required spring isolators sized for the expected load they would carry. Calculations were made to insure that the natural frequency of the isolator would be 3 to 10 times lower than the driving force frequency of the pumps. If the natural frequency was any less the vibration transmitted could be amplified instead of reduced. Isolators of the proper natural frequency were chosen and installed, modifying the stand legs where necessary to keep pump and pipe mating flanges at the correct elevations.

In addition to vibration isolation the following actions were taken to reduce pump vibration:

1. Alignment of pump and motor shafts utilizing a dial gage provided greatly increased accuracy in shaft alignment. The prior method of alignment was by straight-edge and line-of-sight. By fixing the dial gage to the pump shaft and rotating it so that the gage measured perpendicular to the circumference of the motor shaft, we were able to align the shafts within 0.005" in all directions. Vertical alignment was obtained by shimming and horizontal alignment obtained by making use of available "slop" in bolt holes. Additional benefits of better shaft alignment will be less wear on flexible couplings and shaft bearings.

2. Replacement of an excessively worn existing pump with a new Gardner Denver vacuum blower. The existing pump had a severely eroded rotor which was a source of excessive vibration and noise. Another blower was rebuilt by the manufacturer who replaced seals and bearings.

The result of these measures is a noticeably reduced vibration level in the dome room where the exhaust pipe is located, and a reduced structural vibration level in the pump room area.

THOMAS DOME INHALATION EXPOSURE CHAMBER NOISE ABATEMENT PROGRAM

Air Supply Blower Noise Reduction

Noise intensity measurements made in previous years in the basement area containing the air supply blower have indicated that the OSHA limits for human exposure have never been exceeded. However, a facility program has been implemented to reduce noise levels to a comfortable level for the personnel working in and around the operating equipment wherever possible. Readings taken around the blower showed a peak of 87 db over the entire frequency span. The blower sound made normal conversation impossible and work around the area uncomfortable. Some experimental insulation of the blower housing, and construction of a small cover that encased the motor caused some noise reduction, but sound levels were still considered too high.

A new enclosure was designed to totally cover the blower. It was built in a box shape out of Styrofoam® core wood panels in sections to fit easily over the various inlet and exhaust piping access points of the blower. The box consisted of three sides

and a ceiling with one side acting as a removable access panel. Once the box assembly was attached to the basement wall, the inside was lined with a lead-backed foam material, as were the ceiling, exhaust baffle, floor, blower base, and blower housing as shown in Figure 59.

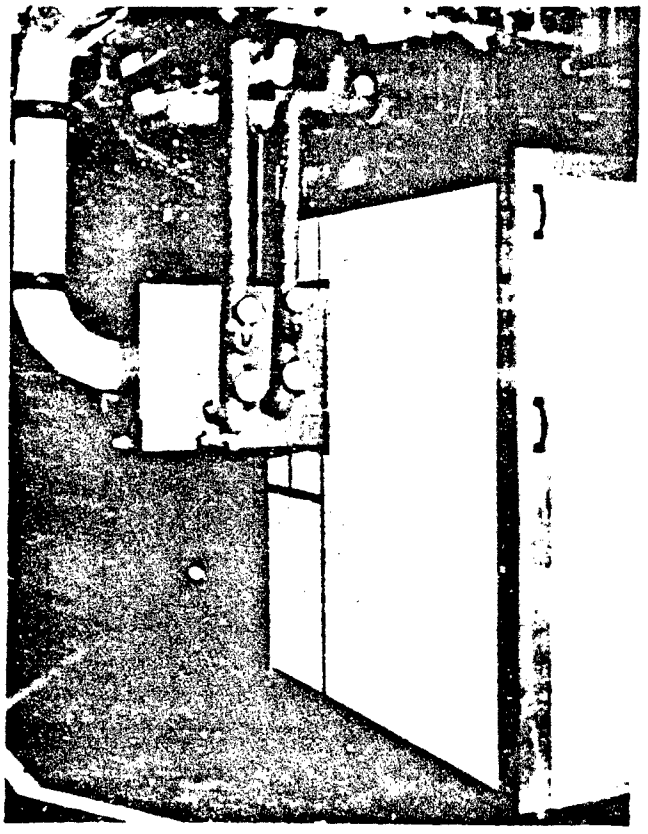
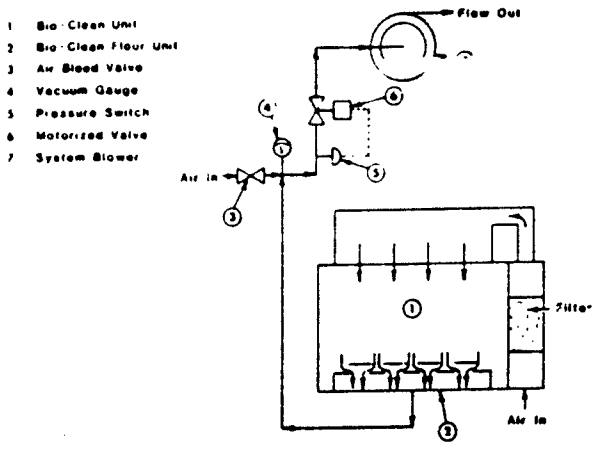


Figure 59. Thomas Dome high air velocity air supply system.

To keep the interior from overheating, two fans, with a total flow capacity of 220 CFM, were installed in the lower left side of the enclosure. This allowed cool air to be pushed past the blower housing and motor to the other side where it exited through an exhaust baffle mounted below the top of the enclosure. Two other boxes also shown in Figure 60 were constructed out of PVC plastic and lined with the lead foam for the inlet air line and exhaust after cooler in order to further seal and insulate the piping.



Schematic of Bio-Clear Exhaust System

Figure 60. Laminar flow booth ventilation system.

Noise intensity measurements taken after installation of the covers showed that the maximum reading was 76.5 db at 500 Hz. This noise reduction permitted normal conversation near the enclosure and reduced noise levels below that generated by all other equipment operating in the basement.

The enclosure cooling system to date has been satisfactory with exhaust temperatures running between 90 - 100°F in the enclosure exhaust while the basement is maintained at 70°F.

The Dome Air Supply Blower sound enclosure design combined efficient sound deadening materials with simple construction to achieve excellent noise reduction without interfering with the operational integrity of the blower unit. The end result was a neat functional unit resulting in reduction of adjacent sound levels and maintaining easy access to the enclosure interior for routine blower maintenance.

Input Valve Noise Reduction

In last year's annual report, remarkable progress in noise reduction was shown in the exposure chambers by the use of an inline muffler coupled with a butterfly valve in place of the older diaphragm valve that was used in the air input lines.

Since then, a new butterfly valve designed especially to reduce noise was installed to further reduce A-weighted noise levels from 69.5 to 65 dBA at 20 CFM and from 79 to 73.6 dBA at 60 CFM flow. Also, further vacuum pump vibration isolation and exhaust pipe insulation was installed which decreased the background noise at zero flow from 67.5 to 60 dBA. These efforts have brought exposure chamber noise down to a level that might occur in an office and have improved the animal environment for the ongoing studies.

VENTILATION SYSTEM FOR LAMINAR FLOW BOOTHS

Laminar flow systems were purchased several years ago to provide clean atmosphere environments for animals treated or held in the Respiratory Toxicology Laboratory and the Toxicology Laboratory in Building 433. Since these were small units, they could easily fit into laboratory areas where daily mixing and dosing of hazardous chemicals could be done in hoods with subsequent transfer of animals to the laminar flow rooms. A problem with this system was that exhaust air was pumped out into the room where human exposure could occur from vaporization of hazardous materials from animal wastes.

To eliminate this potential hazard, systems were designed and constructed to make the units single-pass modules exhausting to outside air. Each exhaust system consisted of four major parts: the floor unit, air bleed valves, pressure activated damper valve, and the system blower. All the major construction was of polyvinylchloride plastic. There were two units modified in Building 433, Room 120, and one in Building 79, Room 122. Figure 60 diagrams the system as presently operating.

The floor unit was designed to fit neatly under the animal racks and was constructed out of PVC plastic plate. The top portion had four rectangular openings that could be adjusted to allow a balanced exhaust flow to occur in the bio-clean enclosure. Constructed in this manner the floor unit did not interfere with daily routines and kept animal feces and other materials from falling into the exhaust system.

The air bleed valves were installed in the duct downstream from the floor unit. The inlets to the air bleed valves were ducted above the ceiling to reduce throttling noises in the working environment. These valves were opened or closed until the

laminar flow room curtains, which covered the opening, became limp. This indicated that a balance between flow in and out existed.

The pressure activated damper valves were designed as safeguards for the exhaust system. They were installed downstream of the bio-clean floor units and air bleed valves and were coupled electrically to pressure switches. As the pressure switches detected header vacuum, they would trigger the motorized damper to open. However, if an equipment failure had occurred to stop flow in the units, these valves would have closed automatically. Having the valves close under these conditions would preclude a down draft stack flow, forcing the air into the laboratory work environment.

The system blowers used were high pressure direct drive types with capacities of 930 CFM, providing laminar flow of at least 160 air changes per hour. An electrical circuit was installed to indicate the blower operation status. This was done by the use of a red/green lighting system which was tied to the pressure switch electrically. When the blower started operating creating a header vacuum, the pressure switch would detect this and activate a green light indicating exhaust flow had occurred. A vacuum gauge was also installed to measure flow and indicate time for filter replacement.

The exhaust system was designed as a simple yet effective means of exhausting chemical vapors and animal odors externally and eliminating human exposure. The systems have been in operation for several months without any major problems.

MULTI-TERMINAL DATA PROCESSING SYSTEM

Intelligent data terminals have been utilized at the THRU Laboratory for some time to provide data-entry capabilities to on-site and off-site time-shared computer systems. The Aeronautical Systems Division (ASD) of the Air Force Systems Command maintained a Control Data Corporation computer system which was accessed by the THRU terminals to provide statistical program support for THRU experiments. These terminals were also utilized to access national databases such as Medline and Toxline services. These terminals had been installed approximately four to five years previously and were composed of 8-bit microprocessor technology. These systems were typically disk based with an

8-bit central processing unit, dual single-density disk drives, 1200 baud modem, 120 character-per-second printer, and 64,000 character random access memory (ram). Several limitations in capability were experienced in the existing systems. These limitations were as follows:

1. The maximum capacity of 8-bit based systems are generally limited to 64,000 character ram capability, and with the single-sided single-density diskettes on the existing systems, character storage on the 8" disks was 241,000 characters. This placed severe restrictions on application involving larger databases.

2. An associated problem involving floppy-disk based systems with minimal capacity is the speed of processing. This can result in an inordinate amount of time required for processing data.

3. The existing systems were composed of hardware from different manufacturers resulting in non-standard operating procedures for different units. This resulted in difficulty in training of personnel when required to operate more than one terminal.

As a solution to the above problems and to progress towards on-site computing capability, in early 1983 a survey was conducted of present technology available in desk-top computers. Several manufacturers were contacted, both on-site and off-site demonstrations were attended and trade shows were visited to evaluate equipment.

An extensive survey was conducted of equipment supplied by several manufacturers. Some of the prime considerations were equipment reliability, company reputation, servicing availability, equipment performance, software, and accessory support. After evaluation of the information the decision was made to procure equipment manufactured by IBM. IBM provides two models which matched the specifications required; the IBM-PC - a floppy disk based unit and the IBM-XT - a hard disk based unit.

These units were configured as shown in Figure 61. The actual parameters and capacities of these units based on the state-of-the-art configurations mentioned above were as follows:

1. 16-bit central processing unit - 8088.
2. Random access memory capacity of either 512 K characters or 640 K characters.
3. Disk storage capacity of either two 360,000 character floppy disks or one 360,000 characters and one 10 megabyte character hard disk.
4. 12" video display terminal both color and monochrome.
5. Detached keyboard.
6. One to three output ports; serial and parallel.
7. Motherboard assembly for five or eight plug-in boards.
8. 200 character-per-second dot matrix printers.
9. 55 character-per-second letter quality printers.
10. 1200 baud programmable modems.
11. 256,000 or 384,000 random access memory boards with output ports and time-of-day clock.
12. Local area network plug-in accessory board.
13. 8-bit microprocessor CPM adaptor plug-in board for 8-bit software compatibility.
14. A plug-in adaptor board providing local area network capability for file transfers between each unit of the multiuser network.

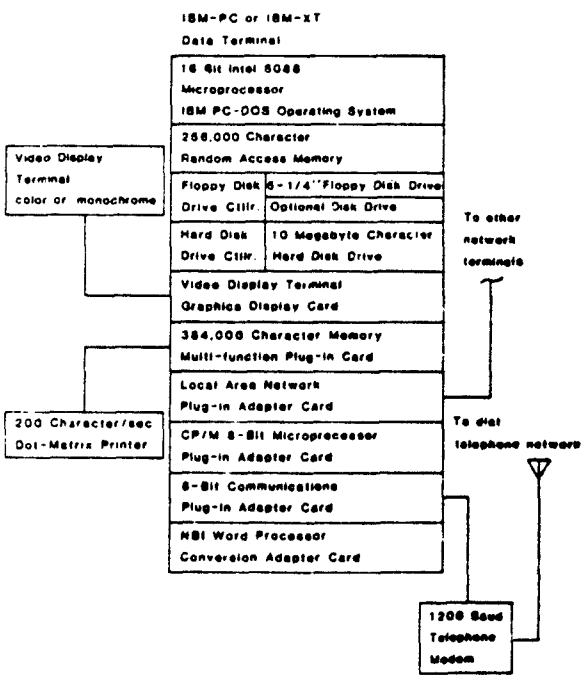


Figure 61. Multi-user computer system terminal.

Accessory and peripheral equipment are supplied by other manufacturers and are compatible with the IBM equipment. After assembly of each of these units it was determined to make the systems available to the appropriate departments at THRU. Data entry facilities at the THRU laboratory had consisted of the central data terminal system used for communication with the large off-site ASD mainframe computer and for local processing of experimental data. Communication was accomplished through a 1200 baud modem. This system had been utilized by several departments of the THRU organization for a number of years.

The new IBM based equipment expands the present system to a multi-station system connected with a networking scheme. This provides multi-user operation and increased storage capacity at each station. Satellite stations have the capability of communication with the local hard disk storage unit or with off-site mainframe computers by means of telephone. Hard copy printout capability also is provided at each station. This system alleviates the delays we had experienced with gaining access to the mainframe computer. It also provides the opportunity to transfer some mainframe data programs to local systems where feasible, resulting in faster turnaround times in reports and improved data security. Software supplied with the system provides centralized control of parts inventories, chamber operating equipment repairs and calibrations, and integration of chamber experimental requirements and equipment availability and maintenance. Significant cost savings will be realized when additional software is provided improving tracking of parts and materials required in operating the THRU experimental chambers.

The system diagrammed in Figure 62 provides a hard disk based data entry system with one central data entry terminal, 35 megabyte hard disk storage capacity and five satellite data entry terminal systems of ten megabyte hard disk capability. The central data entry terminal includes a cartridge tape back-up providing security for critical data stored on the hard disks. This capability also allows for the maintenance of a library of specialized applications software and the storage of experimental data for later retrieval, review, or analysis. The six data entry terminals are connected with a networking scheme allowing the transfer of data between units. Each data entry terminal is supplied with a printer for hard copy printouts of data and 1200 baud modem for communication with an off-site time-sharing computer system. Operating software supplied with the system includes the following applications.

1. Word Processing Package
2. Data Base Manager
3. Spreadsheet Program
4. Spelling Dictionary
5. Communications Package
6. Basic Language Interpreter with Graphics Package.

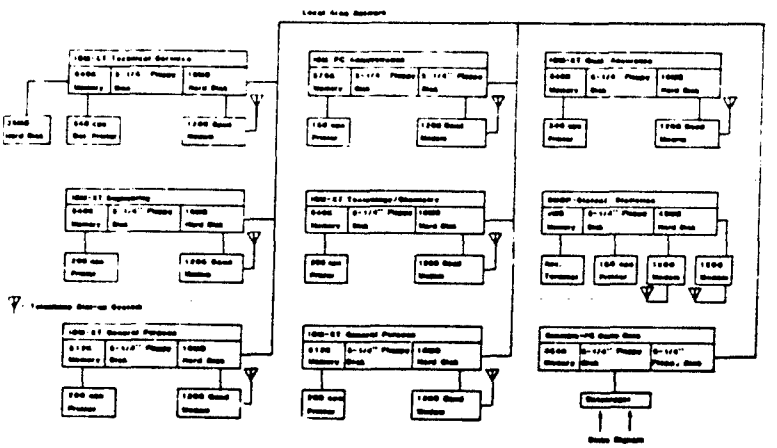


Figure 62. Multi-user terminal system.

This software provides the nucleus of an integrated management information retrieval system allowing improved access to and control of experimental and maintenance activities at the THRU laboratory. Additional applications software is available for specialized requirements or the units may be programmed for custom applications.

The six basic stations of the multi-user terminal system are as follows:

1. Engineering System
2. Technical Services System
3. Chemistry/Toxicology System
4. Administration System
5. Two General Usage Systems

The initial four terminals are located in the areas served for the most efficient utilization of the systems. The engineering system is utilized by engineering personnel to prepare

programs in support of the other terminals in the system and to write engineering programs in support of engineering projects.

The location for the Technical Services data terminal provides centralized access for the control and processing of laboratory data. The Technical Services data clerk is responsible for data input and output to and from the central base computer. These data include information on animal mortality, body weights, and blood chemistry for all experimental animals. In addition, operation of a Beckman Toxsys Computerized Animal Weighing System is also managed. The Chemistry/Toxicology data terminal is utilized for entering technical reports, transmitting technical data to a mainframe computer and for local statistical analysis and printouts. An additional data terminal is located in the Administrative Offices in Building 79. This unit is used by the principal investigator for reviewing and editing reports, accessing national time-shared databases such as Toxline, Chemline and Medline, and for local data processing functions. In addition, it is provided with a 1200 baud modem and a high-speed direct wire connection to an NBI stand-alone word processor. This connection provides high speed transfer of text files between the Administrative and Word Processing areas. A terminal with a basically similar configuration performs the function of collecting environmental data from the exposure chambers. Data are collected and hourly averages are stored on floppy disks for later printout of daily reports of chamber operation. Parameters stored and printed are flow, pressure, dry bulb, wet bulb, and relative humidity. An algorithm built into the terminal program calculates relative humidity from the dry bulb and wet bulb temperature inputs.

There are two additional data terminals in use at THRU that are located too distant to be connected to the hard-wired network. These terminals are located in the Statistics Department area, Building 29, and the Quality Assurance and Safety Department area in Building 433. Both of these data terminals are able to communicate with the other terminals by way of a communications program, modem, and the dial telephone network system.

The Statistics data terminal (Statcat), diagrammed in Figure 63 is a specialized microcomputer system based on a 32 bit microprocessor, the Motorola 68000. The unit is equipped with a 600 K floppy disk, 43 megabyte hard disk and 2 megabytes of random access memory. The system software is a Unix V based operating system capable of running the BMPD set of statistical programs. Statistical analyses of experimental data are now processed by

BMPD programs running on the ASD mainframe computer. It is planned to transfer these programs to the Statcat, resulting in enhanced control of experimental data and reducing delays encountered in utilizing the mainframe computer system. The other remotely located data terminal is located in the Quality Assurance and Safety Department in Building 433. The data terminal is equipped similarly to the other terminals. The system will be utilized for storage and retrieval of events schedules, GLP auditing of stored data, local data processing, and time shared terminal functions with the mainframe ASD computer.

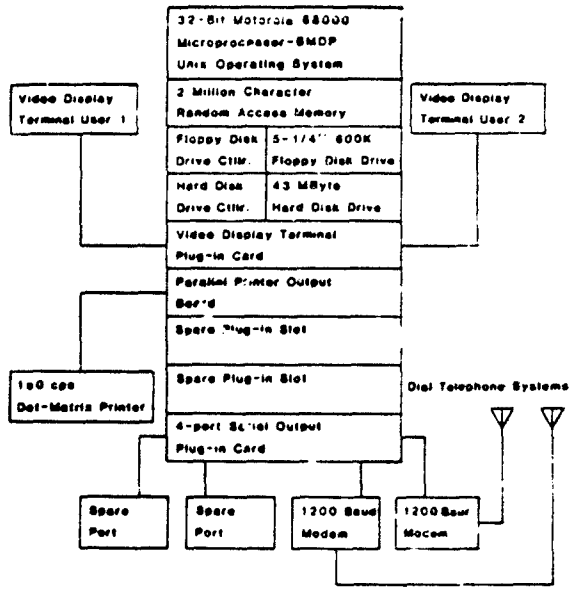


Figure 63. Statistics data terminal diagram.

TECHNICIAN TRAINING PROGRAMS

Animal Technicians

The current status of the UCI animal technicians regarding certification in the AALAS program is as follows:

6 - Laboratory Animal Technologist
3 - Laboratory Animal Technician
1 - Assistant Animal Technician

The basic course outline of certification by AALAS was described in detail in a previous report (MacEwen and Vernot, 1975). All references listed by AALAS utilized in preparing for examinations are now available through the UCI and Air Force libraries.

Several technicians have been trained in observing and scoring test chickens for neurotoxic signs and symptoms. The animal technicians have been actively involved in the dosing and scoring during the intratracheal injection techniques.

Three animal technicians have been responsible for the presentation of a total of seven posters at district and national AALAS conferences. Two additional posters have been submitted for presentation at the 1984 national AALAS conference.

A scientific article entitled "A Simple Device for Intratracheal Injections in Rats" was published in the Laboratory Animal Science Journal, Volume 32, No. 5, October 1982. This article received international recognition as over 60 reprint requests were received from Europe, Asia, Africa, and South America.

Two animal technicians were accepted to the AALAS Foundation sponsored technician exchange program held at the Laboratory Animal Research Center of the Rockefeller University in New York City. The purpose of the exchange is to provide competent technicians with new work experiences.

Special Performance Awards were received by the principal and senior animal technician for excellence in job performance. The prinicipal animal technician was named "Technologist of the Year" at the 1983 District V AALAS meeting in Indianapolis, Indiana. He was recommended for the award by both UCI and Air Force management.

Additional training programs have been made available for training new animal technicians and as refresher courses for experienced technicians. The following is a list of all programs being utilized:

Purina Animal Care
Animal Care Training Videotapes
Advanced Practical Training
Laboratory Animal Medicine and Audiotutorial Series
National Laboratory Animal Education Exchange

Chamber Technicians

Since the last Annual Report the Thomas Dome Standard Operating Procedures have been revised to accomodate facility equipment changes. In addition, many of the special fire emergency procedures have been eliminated since the facility no longer operates with 100% oxygen and contaminant generation systems are routinely fitted with automatic safety devices. A basic laboratory fire emergency procedure still exists in the Thomas Dome Standard Operating Procedure manual, however.

During the year all Chamber Technicians received individual light-weight respiratory protection hoods equipped with wireless communication systems. Personal systems for each technician allow for closer attention to maintenance of these important safety devices. Technicians entering the chambers for animal maintenance also wear disposable Tyvek® or polyethylene coated Tyvek® suits with elastic wrists and cuffs, extended cuff rubber gloves, and shin high anti-skid boots.

The Thomas Dome Standard Operating Procedures Training Program for new technicians has been revised to accommodate the SOP changes. The subjects covered in the training program for new technicians are as follows:

I. Orientation

A. Laboratory Mission

B. Job Responsibilities

1. General coverage of SOP's.
2. General coverage of lab operations.

C. Personnel Responsibilities

1. GLP procedures.

II. Standard Operation of Chambers

A. Observer "A" Normal Routine

1. Dome start-up.
2. Establish flow.
3. Normal readings.
4. Dome entry operation.

B. Observer "B" Checklist

C. Dome Entrant Duties

1. Dome entry operation.
2. Dome cleaning and cage changes.

D. Dome Cap Raising and Lowering

III. Mechanical Equipment

A. Vacuum Pump Failure

1. Facility A pump.
2. Facility B pump.
3. Observer duties.

B. Air Compressor Failure

1. Main air compressor.
2. Back-up air compressors.
3. Air dryers.
4. Observer duties.

C. Complete Power Failure

1. Facility A procedures.
2. Facility B procedures.
3. Observer duties.

D. Air Supply Fan Failure

1. Main air supply fan.
2. Back-up supply fan.
3. Observer duties.

E. Waste Catch Tank Draining

1. Transfer-dome to tank.
2. Emptying of tank.

F. TOXSYS System Data Collection SOP

IV. Emergencies

A. Fire in Exposure Laboratory

1. Observer A duties and options.
2. Observer B duties and options.

B. Rescue of Incapacitated Dome Entrant

1. Rescue criteria.
2. Observer A duties.
3. Observer B duties.

C. Operation of Scott Air Pak (SCBA)

1. Criteria for use.
2. Procedures.

D. Building 429 Alarm

1. Observer A duties.

The monthly emergency training procedures program has also been revised. Training in the procedures is conducted by the Senior Technicians on each shift. Periodic written examinations are given by the Principal Technician to all Chamber Technicians. Revisions of any procedure and/or retraining is made by the Principal Technician as the need arises. Listed below is the schedule for the training procedures and examinations given during the past year. Documentation of all practical, oral, and written examinations is maintained by Principal Technician.

Date	Procedure
January	- The care and operation of the Scott Air Pak and the dual element cartridge type respirators.
February	- Vacuum pump failure.
March	- Air compressor failure.
April	- Air supply fan failure.
May	- Complete power failure.

June	- Rescue of an incapacitated dome entrant.
July	- Building 429 alarm.
August	- Fire in exposure laboratory area.
September	- GLP procedures and toxicology SOP's.
October	- Vacuum pump failure.
November	- Air compressor failure.
December	- Complete power failure.

Written examinations

It has been the goal of the THRU to involve all technicians in the certification program sponsored by the American Association of Laboratory Animal Science (AALAS). The Chamber Technician group presently has nine individuals certified in the AALAS program. The tenth technician is a new hire and should obtain certification within the next year. The levels of AALAS certification are shown below:

Laboratory Animal Technologists	2
Laboratory Animal Technicians	4
Assistant Laboratory Animal Technicians	3

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